Anti-inflammatory Therapy and Cerebrospinal Fluid Diagnosis in Alzheimer's Disease

Daniëlle de Jong

The research described in this thesis was supported in part by grants from Zon-MW Innovational Research (no. 917.46.331), 'Hersenstichting Nederland', 'Alzheimer Nederland', and the American Health Assistance Foundation (A2001-15).

Cover design: Maan vormgevers b.v., Ulvenhout
Photo: A.H.M. de Jong
Printed by: Koninklijke Broese en Peereboom b.v., Breda

ISBN 978-90-9025634-4

The publication of this thesis was financially supported by:
The Department of Neurology (Radboud University Nijmegen Medical Centre) and UCB Pharma b.v.

Anti-inflammatory therapy and cerebrospinal fluid diagnosis in Alzheimer's disease

Een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

Proefschrift

ter verkrijging van de graad van doctor
aan de Radboud Universiteit Nijmegen
op gezag van de rector magnificus, prof. mr. S.C.J.J. Kortmann,
volgens besluit van het College van Decanen
in het openbaar te verdedigen op dinsdag 21 september 2010
om 15.30 uur precies

door

Daniëlle de Jong
geboren op 31 juli 1971
te Eindhoven

Promotor

Prof. dr. H.P.H. Kremer (Universitair Medisch Centrum Groningen)

Copromotores

Dr. R.W.M.M. Jansen (Medisch Centrum Alkmaar)

Dr. ir. M.M. Verbeek

Manuscriptcommissie

Prof. dr. F. Verhey (Academisch Ziekenhuis Maastricht)

Prof. dr. M.G.M. Olde Rikkert

Prof. dr. P. Smits

Voor mijn vader,
overleden op 31 december 2009,
vlak na het inleveren van dit manuscript.

Table of contents

Chapter 1

General introduction, aims and outline of this thesis

General introduction

Dementia, a syndrome of brain dysfunction, has many possible causes. Alzheimer's disease (AD) is the most common cause of dementia.(1) It is a progressive neurodegenerative disorder that is characterized neuropathologically by the deposition of amyloid β protein (Aβ) -containing extracellular amyloid plaques, intracellular neurofibrillary tangles (NFTs), and neuronal loss. AD patients gradually develop memory loss and other cognitive deficits, such as aphasia, apraxia, agnosia, and impaired executive functioning. Also, neuropsychiatric disturbances such as personality changes, depression, psychosis, anxiety, and agitation are common in AD patients. (2) It has a devastating effect on the life of patients, and places a great burden on their caregivers.

AD affects millions of individuals worldwide, and its prevalence increases with age.(3). The ageing of populations in most western world countries implies that the number of patients will increase even further in the near future, with great socio-economic consequences for western societies. So far there are no drugs available that prevent, cure or slow the progression of the disease. The currently available treatments such as cholinesterase inhibitors (e.g. rivastigmine, galantamine, donepezil), and memantine, an N-methyl-D-aspartate receptor antagonist, only offer a moderate symptomatic effect.(4)

Since 1982, a growing number of investigators has studied the role of inflammation in the pathogenesis of AD, and the consequent hypothesis that AD can be treated with anti-inflammatory drugs.(5-7) Through extensive neuropathological, epidemiological, and animal research evidence has been compiled that an inflammatory process actively mediates AD pathology, and that the use of anti-inflammatory drugs, in particular non-steroidal anti-inflammatory drugs (NSAIDs), may lower the risk of developing AD.(8-10) However, there is still insufficient evidence that NSAIDs can actually cure or slow the progression of AD; Clinical trials have failed to prove that NSAIDs have a neuroprotective effect in this disease.

When conducting a clinical trial testing an NSAID in AD patients, there are two important issues to consider. First, it is crucial to choose the most appropriate NSAID to study, since the mechanism of action differs between the various NSAIDs, especially regarding AD pathology.(11-13) Also, the selected NSAID should be able to

cross the blood-brain barrier. Second, it is important to include the proper patients in clinical trials. AD patients should be distinguished carefully from patients with other dementia types such as vascular dementia, frontotemporal lobar degeneration, and dementia with Lewy bodies, since all dementia types have a different pathogenesis and a different clinical course. An accurate AD diagnosis is not only necessary for research purposes, but also essential for appropriate support and (symptomatic) treatment of AD patients. However, with the current clinical diagnostic criteria it is still impossible to adequately discriminate between the different dementias.(14;15) Other biomarkers, such as the quantification of brain-specific proteins in cerebrospinal fluid (CSF), may help to improve this selection.

Aβ protein is the major component of amyloid plaques, and tau protein the primary constituent of NFTs in brains of AD patients. Therefore, these proteins were first regarded as potential diagnostic biomarkers in CSF of dementia patients. Decreased concentrations of $A\beta_{42}$, and increased concentrations of total tau protein, which includes both normal and hyperphosphorylated tau, were found in CSF of AD patients compared to controls.(16;17) Different levels of these proteins were found in other dementia types compared to AD, which improves the discrimination between different dementia types.(18-20) However, additional CSF biomarkers and novel technologies are necessary to further improve this discrimination.

Aims and outline of this thesis

The content of this thesis is divided in two coherent parts. The first part of this thesis concerns the treatment of Alzheimer disease (AD), especially with nonsteroidal anti-inflammatory drugs (NSAIDs). The second part relates on the diagnosis of AD using biomarkers in cerebrospinal fluid (CSF).

Alzheimer's disease and anti-inflammatory drugs

The aim of this part of the thesis was to review the role of inflammation in the pathogenesis of AD, and to study the effect of one NSAID, indomethacin, on the progression of AD. In addition, the external validity of randomized controlled trials (RCTs) with AD patients was explored.

In **chapter 2.1** we review different lines of research that address AD and inflammation. We reviewed which NSAID is the best candidate to study in a clinical trial with AD patients; indomethacin appeared to be one of the most promising NSAIDs. The methods and results of a double-blind, randomized, placebo-controlled trial with indomethacin in AD patients are described in **chapter 2.2**. Furthermore, we investigated the characteristics of all patients that participated in our trial, and compared them with all remaining AD patients seen at our memory clinic for diagnosis and treatment during the four-year recruitment period of the trial. This study of the external validity of the results of our trial is presented in **chapter 2.3**. Also, 72 RCTs with AD patients testing various drugs were selected from the literature and reviewed for further comparisons.

Cerebrospinal fluid diagnosis in Alzheimer's disease

The aim of this part of the thesis was to investigate whether the analysis of biomarkers, especially in CSF, may be helpful in discriminating AD from other dementias.

In **chapter 3.1** we describe the results of the analysis of CSF levels of total tau protein, amyloid β_{42} protein, and tau phosphorylated at threonine 181 in AD patients compared to patients with vascular dementia. The diagnostic value of another CSF biomarker, neurofilament (NF) protein, is described in **chapter 3.2**. We investigated the diagnostic value of NF analysis to discriminate in relatively young dementia patients between frontotemporal lobar degeneration and early-onset AD, and in

elderly dementia patients between dementia with Lewy bodies and late-onset AD. **Chapter 3.3** is a review of the literature regarding neurochemical biomarkers for AD. We summarize the current state-of-the-art of biomarkers for AD in CSF and in plasma.

In **chapter 4.1**, the main findings of the thesis are summarized followed by a discussion and recommendations for future research.

References

(1) Jorm AF, Jolley D. The incidence of dementia: a meta-analysis. Neurology 1998 Sep; 51(3):728-733.

(2) Mega MS, Cummings JL, Fiorello T, Gornbein J. The spectrum of behavioral changes in Alzheimer's disease. Neurology 1996 Jan;46(1):130-135.

(3) Ritchie K, Kildea D, Robine JM. The relationship between age and the prevalence of senile dementia: a meta-analysis of recent data. Int J Epidemiol 1992 Aug;21(4):763-769.

(4) Raina P, Santaguida P, Ismaila A, et al. Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline. Ann Intern Med 2008 Mar 4;148(5):379-397.

(5) Eikelenboom P, Stam FC. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta Neuropathol Berl 1982;57(2-3):239-242.

(6) McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21(2): 195-218.

(7) Breitner JC. The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease. Annu Rev Med 1996;47(s).

(8) McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging 2001 Nov;22(6):799-809.

(9) in 't Veld, Ruitenberg A, Hofman A, et al. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 2001 Nov 22;345(21):1515-1521.

(10) Lim GP, Yang F, Chu T, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 2000 Aug 1;20(15):5709-5714.

(11) Combs CK, Johnson DE, Karlo JC, Cannady SB, Landreth GE. Inflammatory mechanisms in Alzheimer's disease: inhibition of beta- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. J Neurosci 2000 Jan 15;20(2):558-567.

(12) Asanuma M, Nishibayashi-Asanuma S, Miyazaki I, Kohno M, Ogawa N. Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. J Neurochem 2001 Mar;76(6):1895-1904.

(13) Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature 2001 Nov 8;414(6860):212-216.

(14) Lopez OL, Litvan I, Catt KE, et al. Accuracy of four clinical diagnostic criteria for the diagnosis of neurodegenerative dementias. Neurology 1999 Oct 12;53(6):1292-1299.

(15) Lopez O, Claassen D, Boller F. Alzheimer's disease, cerebral amyloid angiopathy, and dementia of acute onset. Aging (Milano) 1991 Jun;3(2):171-175.

(16) Galasko D. Cerebrospinal fluid levels of A beta 42 and tau: potential markers of Alzheimer's disease. J Neural Transm Suppl 1998;53:209-221.

(17) Hulstaert F, Blennow K, Ivanoiu A, et al. Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 1999 May 12;52(8):1555-1562.

(18) Andreasen N, Minthon L, Davidsson P, et al. Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol 2001 Mar;58(3):373-379.

(19) Kanemaru K, Kameda N, Yamanouchi H. Decreased CSF amyloid beta42 and normal tau levels in dementia with Lewy bodies. Neurology 2000 May 9;54(9):1875-1876.

(20) Riemenschneider M, Wagenpfeil S, Diehl J, et al. Tau and Abeta42 protein in CSF of patients with frontotemporal degeneration. Neurology 2002 Jun 11;58(11):1622-1628.

Chapter 2

Alzheimer's disease and anti-inflammatory drugs

Chapter 2.1

The possible suppression of Alzheimer's disease by nonsteroidal anti-inflammatory drugs (review)

D. de Jong
R.W.M.M. Jansen
M.M. Verbeek
H.P.H. Kremer

Adapted from: Ned Tijdschr Geneeskd 2002 Nov 2;146(44):2074-2078

Abstract

Ever since inflammatory mediators were detected surrounding amyloid plaques in brains of patients with Alzheimer's disease (AD), researchers have been interested in the role of inflammatory mechanisms in AD and its potential treatment with anti-inflammatory drugs. Epidemiological studies have already demonstrated that use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a reduced risk for the development of AD. The effect of NSAIDs in AD is probably mediated by activation of the peroxisome proliferator-activated receptor-γ. Administration of NSAIDs to AD mice suppressed plaque formation and inflammation. Together, these findings raised the suggestion that NSAIDs will be able to retard AD progression. So far, only one small clinical trial has shown that treatment with NSAIDs significantly delayed cognitive decline in AD patients. Large randomized double-blind placebo-controlled trials are needed to demonstrate a definite beneficial effect of NSAIDs in AD.

Introduction

Since 1982, when complement factors were found present in amyloid plaques in brains of patients with Alzheimer's disease (AD), the role of inflammation in the pathogenesis of this disease gained interest.(1) In 1990, the results of epidemiological studies in patients with rheumatoid arthritis suggested that the prolonged use of anti-inflammatory drugs might have a protective effect on incipient AD (primary prevention).(2) This generated the hypothesis that anti-inflammatory drugs could also have a therapeutic effect on patients already suffering from AD (secondary prevention). In 1993, the positive results of a small double blind placebo-controlled trial that tested the effect of indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), in AD patients supported this hypothesis.(3)
Ever since, many studies have been published that investigated inflammation and anti-inflammatory therapy in AD. However, a significant effect of an anti-inflammatory drug in a randomized controlled trial had not yet been found. As all the currently registered drugs for AD, co-dergocrine, rivastigmine, galantamine, and memantine, have only a limited symptomatic effect, the hypothesis that the use of inexpensive anti-inflammatory drugs can prevent or retard the progression of AD remains attractive.

In the following paragraphs the inflammatory hypothesis, and the clinical applicability of the most promising anti-inflammatory drugs in AD, the NSAIDs, will be discussed.

Neuropathological data

The most characteristic neuropathological changes in the brains of AD patients are amyloid plaques, that consist of extraneuronal accumulations of amyloid-β (Aβ) protein, and neurofibrillairy tangles, formed by intraneuronal deposits of tau protein. The numbers of plaques and tangles have been found to correlate significantly with disease severity.(4) They probably cause neuronal dysfunction and cell death, that eventually leads to clinical symptoms of the disease. As plaques and tangles are foreign and disruptive elements in brain tissue, they may initiate an immuno-

logical reaction. However, granulocytes and lymphocytes, elements of the classical acute immunological response, are conspicuously absent. Instead, amyloid plaques are surrounded by macrophage-like cells called microglia. In vitro research demonstrated that microglia are activated by Aβ protein present in amyloid plaques. Activated microglia appear to play a role in the transformation of diffuse amyloid plaques, also present in some non-demented elderly persons, into neuritic amyloid plaques. (5) In addition, activated microglia produce proteins that play a role in inflammation, like complement factors, proinflammatory cytokines and chemokines (interleukin (IL)-1, IL-6, transforming growth factor (TGF)-β and tumor necrosis factor (TNF)-α). They also generate primary neurotoxic products such as free radicals and nitric oxide (NO).(6) Not only the products produced by microglia, but also other inflammatory mediators can be found in and around plaques and tangles (table 1).(7) The resulting inflammatory response causes tissue damage; and this again may be pro-inflammatory in itself.

Table 1

Inflammatory mediators in Alzheimer's disease	
Acute phase proteins	C-reactive protein, α1-antichymotrypsin, α2-macroglobulin, amyloid P component
Adhesion molecules	Intercellular adhesion molecules (ICAM-1 and ICAM-2)
Complement factors	C1q, C2, C3, C4, C5, C6, C7, C9, C5b-9
Complement inhibitors	C1 inhibitor, C4-binding protein
Cytokines and chemokines	Interleukin-1α, interleukin 6, tumor necrosis factor-α, transforming growth factor-β

Epidemiological data

If inflammation plays a role in the development or progression of AD, prolonged use of anti-inflammatory drugs should lower the risk of AD. This hypothesis has been tested in many epidemiological studies. Despite differences in design and methods of these studies, they all show a remarkable consistent picture of an association between the use of NSAIDs and a lower risk of AD (table 2).

Table 2

Risk of Alzheimer's disease while using non-steroidal anti-inflammatory drugs (NSAIDs); results of epidemiological research

Study	Publication year	Design	OR/RR (95% CI)	Duration NSAID use
McGeer (8)	1996	Case-control	0,50 (0,34 - 0,72)	variable
Stewart (9)	1997	Prospective	0,40 (0,19 - 0,84)	> 2 years
Beard (10)	1998	Case-control	0,79 (0,45 - 1,38)	variable
In 't Veld (11)	1998	Case-control	0,68 (0,21 - 2,14)	> ½ year
Anthony (12)	2000	Case-control	0,47 (0,24 - 0,90)	variable
In 't Veld (13)	2001	Prospective	0,20 (0,05 - 0,83)	> 2 year

OR/RR = odds ratio or relative risk; CI = confidence interval

In 1996, a meta-analysis of 17 epidemiological studies was published investigating the use of anti-inflammatory drugs by patients with arthritis, and their risk for AD: 14 were case-control studies.(8) Pooling seven of these studies with anti-inflammatory drug use as risk factor, an odds ratio (OR) of 0,556 ($p < 0,0001$) was found. For four studies with steroids the OR was 0,656 ($p = 0,049$), and for three studies with NSAIDs the OR was 0,496 ($p = 0,0002$).(8)

Results of epidemiological studies published after 1996 supported these initial findings.(9-13) Furthermore, an additionally decreased risk of AD was found with prolonged versus short term use of NSAIDs, especially among those with two or more years of NSAID use.(9;11;13) In the most recent prospective study the relative risk of AD was 0,95 (95 % CI, 0,70 – 1,29) in subjects with short-term use of NSAIDs (one month or less of cumulative use).(13) In those with intermediate-term use (more than 1 but less than 24 months), risk was 0,83 (95 % CI, 0,62 – 1,11), and 0.20 (95 % CI, 0.05 to 0.83) in those with long-term use (24 months or more). No significant risk reduction was found in long-term users of salicylates.(13)

Mechanism of action of NSAIDs

Inhibition of cyclooxygenase (COX), responsible for the conversion of arachidonic acid into inflammatory mediators such as prostaglandins, was considered the most important mechanism of action of NSAIDs. The two COX iso-enzymes, COX-1 and COX-2, are both inhibited by classical NSAIDs. Inhibition of COX-2 seems mainly responsible for the anti-inflammatory effect. However, the effects of NSAIDs can not be solely of mainly ascribed to inhibition of COX, since anti-inflammatory effects will only be reached at much higher doses than necessary for the inhibition of COX. This suggests another mechanism of action.(14)

An alternative mechanism of action of NSAIDs may be the activation of the peroxisome proliferator-activated receptor-gamma (PPAR-γ). Since 1997 the role of PPAR-γ in inflammation, and the effect of NSAIDs on this receptor, has received much interest. PPAR-γ is a nuclear receptor that, when activated, yields an anti-inflammatory effect. Most classical NSAIDs, such as indomethacin, naproxen, and ibuprofen are agonists of PPAR-γ, with the exception of diclofenac, which is only a partial agonist.(15;16) It has been demonstrated that PPAR-γ agonists inhibit the Aβ-stimulated activation of microglia, and thus prevent the excretion of pro-inflammatory and directly neurotoxic products.(17) Also, PPAR-γ agonists appear to reduce the NO mediated apoptotic cell death by inhibiting inducible NO-synthetase.(18) Contrary to COX-inhibition, activation of PPAR-γ can explain previous findings such as the in vitro suppression of the production of pro-inflammatory cytokines (IL-1 and IL-6), and the reduction of NO-production and apoptosis by NSAIDs.(19;20) It also explains the previously demonstrated suppression of microglia activation in postmortem brain tissue of elderly non-demented individuals who were chronically exposed to anti- inflammatory drugs.(21)

Another potential mechanism of action of NSAIDs is the reduction of $A\beta_{42}$ formation and the inhibition of the formation of neuritic amyloid plaques. $A\beta_{42}$ is the 42 amino-acid long easily aggregating form of Aβ protein that, in contrast to the isoforms $A\beta_{38}$ and $A\beta_{40}$, is mainly held responsible for the initiation of neuritic amyloid plaques. In vitro research has demonstrated that the NSAIDs ibuprofen and indomethacin not only lower the amount of $A\beta_{42}$, but also increase the amount of the less aggregating isoform $A\beta_{38}$. This may be caused by an effect on the action

of γ-secretase, one of the enzymes responsible for the formation of Aβ from the amyloid precursor protein (APP). However, not all NSAIDs exert this effect. Naproxen, meloxicam (a non-selective COX-2 inhibitor), SC-560 (a selective COX-1 inhibitor), and salicylic acid were not able to suppress $A\beta_{42}$ secretion.(22)

Animal models

Animal models appear to offer the most pertinent model systems to test the effects of anti-inflammatory drugs. The frequently used transgenic mouse model Tg2576 overexpresses a mutant form of APP, causing an elevated production of $A\beta_{40}$ and $A\beta_{42}$. These animals develop age-related neuritic amyloid plaques, and impairment in learning and memory. Administration of ibuprofen during six months to Tg2576 mice reduced the amount of Aβ deposits, the number of activated microglia, and IL-1β production.(23) Specifically, it was the amount of $A\beta_{42}$ that was decreased.(22)

Also, studies in non-transgenic animal models supported the efficacy of NSAIDs. Chronic infusion of soluble Aβ during two weeks in the lateral ventricles of rats caused extraneuronal depositions of Aβ, and the activation of microglia that surround the ventricles. This Aβ induced microglial activation was significantly attenuated in animals receiving concurrent intravenous treatment with indomethacin.(24)

Clinical data

The ultimate proof of the clinical relevance of the inflammatory hypothesis and the efficacy of NSAIDs on the development and progression of AD can only be provided by placebo-controlled clinical trials. Until 2002, only five clinical trials had been published.

In the first double-blind, placebo-controlled trial published in 1993, 44 AD patients were treated with either placebo or 100-150 mg/day indomethacin during a 6 month period.(3) When the results of the Alzheimer's Disease Assessment Scale (ADAS), Mini-Mental State Examination, Boston Naming Test and Token Test were combined in an aggregate score, patients treated with indomethacin had improved

1,3%, and patients receiving placebo had declined 8,4%. However, the results of the individual cognitive tests appeared not significantly different between groups. Combined with a large percentage of drop-outs (16 patients), the interpretation of the results of this small study is difficult.

In a second pilot trial in 41 AD patients, diclofenac was combined with misoprostol.(25) After six months a nonsignificant trend was found for patients in the placebo group to have deteriorated more than diclofenac/misoprostol-treated patients, as measured on the cognitive subscale of the ADAS (ADAS-cog). The small group of patients, together with a relatively large withdrawal rate (14 patients), may explain the non-significant results of this study.(25)

In 2000 the results of a third trial with prednisone in 138 AD patients were published.(26) After one year of treatment, no difference in cognitive decline (ADAS-cog) between groups could be found. However, the results of this study are potentially obscured by the occurrence of behavioral problems in the prednisone group.(26)

The results of a fourth trial with the selective COX-2 inhibitor celecoxib in 425 AD patients were presented in 2000 (S.M. Sainali, written communication, 2000). After one year of treatment, cognitive decline (ADAS-cog) in the celecoxib and the placebo group was not significantly different.

In the fifth trial, the antimalarial drug with anti-inflammatory qualities hydroxychloroquine was compared to placebo.(27) Again, after 18 months cognitive decline (ADAS-cog), as well as decline in activities of daily living, was similar in both groups. (27)

Appropriate NSAIDs for further trials in AD

If one intends to study whether NSAIDs can retard the cognitive decline in AD, the selection of the NSAID to be investigated becomes crucial. Pharmacological and pharmacokinetic properties then take center stage (table 3). As COX-inhibition by NSAIDs appears not to play an important role, the choice of a selective COX-2 inhibitor is not obvious, despite the favorable side-effects profile. Decreasing the production of $A\beta_{42}$ through an effect on γ-secretase also seems irrelevant; this would only have a

primary preventive effect, i.e. when the neuritic plaques still have to be formed. The preferred NSAIDs have agonistic effects on PPAR-γ, since it is expected that they will be able to retard the progression of AD.

Table 3

Properties of various non-steroidal anti-inflammatory drugs

Drug	COX-inhibition	PPAR-γ agonist	Effect on Aβ production	Blood-brain barrier passage
Salicylic acid	COX-1	?	no	yes
Celecoxib	COX-2	?	no	?
Diclofenac	COX-1 + 2	partial	?	yes
Ibuprofen	COX-1 + 2	yes	yes	yes
Indomethacin	COX-1 + 2	yes	yes	yes
Naproxen	COX-1 + 2	yes	no	yes

COX = cyclooxygenase; PPAR-γ = peroxisome proliferator-activated receptor-gamma; Aβ = amyloid-β protein; ? = unknown.

The classical NSAIDs ibuprofen, indomethacin, and naproxen are PPAR-γ agonists, they pass the blood-brain barrier, and moreover they are cheap.(28) Based on the present information, one of these NSAIDs would be preferred as investigational drug in AD patients. However, an important disadvantage will be the expected side-effects, especially the gastro-intestinal side-effects. These may be countered by adding a proton pump inhibitor or misoprostol, but this increases the treatment costs.

Therefore, we started a randomized double-blind placebo-controlled clinical trial with indomethacin and the proton pump inhibitor omeprazole in 160 AD patients at the Radboud University Nijmegen Medical Center, and at the Rijnstate Hospital, Arnhem, The Netherlands. In the United States of America, a similar study is being conducted with naproxen and the COX-2 selective NSAID rofecoxib. Hopefully, the results of these studies will provide answers to the question whether NSAIDs have a therapeutic effect in AD, and which NSAID is the most appropriate.

References

(1) Eikelenboom P, Stam FC. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta Neuropathol Berl 1982;57(2-3):239-242.

(2) McGeer PL, McGeer E, Rogers J, Sibley J. Anti-inflammatory drugs and Alzheimer disease. Lancet 1990 Apr 28;335(8696):1037.

(3) Rogers J, Kirby LC, Hempelman SR, et al. Clinical trial of indomethacin in Alzheimer's disease. Neurology 1993;43(8):1609-1611.

(4) Cummings BJ, Pike CJ, Shankle R, Cotman CW. Beta-amyloid deposition and other measures of neuropathology predict cognitive status in Alzheimer's disease. Neurobiol Aging 1996 Nov;17(6):921-933.

(5) Mackenzie IR, Hao C, Munoz DG. Role of microglia in senile plaque formation. Neurobiol Aging 1995 Sep;16(5):797-804.

(6) Hemmer K, Fransen L, Vanderstichele H, Vanmechelen E, Heuschling P. An in vitro model for the study of microglia-induced neurodegeneration: involvement of nitric oxide and tumor necrosis factor-alpha. Neurochem Int 2001 Jun;38(7):557-565.

(7) McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. Neurobiol Aging 2001 Nov;22(6):799-809.

(8) McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology 1996;47(2):425-432.

(9) Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997;48(3):626-632.

(10) Beard CM, Waring SC, O'Brien PC, Kurland LT, Kokmen E. Nonsteroidal anti-inflammatory drug use and Alzheimer's disease: a case- control study in Rochester, Minnesota, 1980 through 1984. Mayo Clin Proc 1998 Oct;73(10):951-955.

(11) in 't Veld, Launer LJ, Hoes AW, et al. NSAIDs and incident Alzheimer's disease. The Rotterdam Study. Neurobiol Aging 1998 Nov;19(6):607-611.

(12) Anthony JC, Breitner JC, Zandi PP, et al. Reduced prevalence of AD in users of NSAIDs and H2 receptor antagonists: the Cache County study. Neurology 2000 Jun 13;54(11):2066-2071.

(13) in 't Veld, Ruitenberg A, Hofman A, et al. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. N Engl J Med 2001 Nov 22;345(21):1515-1521.

(14) Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--differences and similarities. N Engl J Med 1991 Jun 13;324(24):1716-1725.

(15) Jaradat MS, Wongsud B, Phornchirasilp S, et al. Activation of peroxisome proliferator-

activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. Biochem Pharmacol 2001 Dec 15;62(12):1587-1595.

(16) Adamson DJ, Frew D, Tatoud R, Wolf CR, Palmer CN. Diclofenac antagonizes peroxisome proliferator-activated receptor-gamma signaling. Mol Pharmacol 2002 Jan;61(1):7-12.

(17) Combs CK, Johnson DE, Karlo JC, Cannady SB, Landreth GE. Inflammatory mechanisms in Alzheimer's disease: inhibition of beta- amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. J Neurosci 2000 Jan 15;20(2):558-567.

(18) Heneka MT, Feinstein DL, Galea E, Gleichmann M, Wullner U, Klockgether T. Peroxisome proliferator-activated receptor gamma agonists protect cerebellar granule cells from cytokine-induced apoptotic cell death by inhibition of inducible nitric oxide synthase. J Neuroimmunol 1999 Dec;100(1-2):156-168.

(19) Bour AM, Westendorp RG, Laterveer JC, Bollen EL, Remarque EJ. Interaction of indomethacin with cytokine production in whole blood. Potential mechanism for a brain-protective effect. Exp Gerontol 2000 Oct;35(8):1017-1024.

(20) Asanuma M, Nishibayashi-Asanuma S, Miyazaki I, Kohno M, Ogawa N. Neuroprotective effects of non-steroidal anti-inflammatory drugs by direct scavenging of nitric oxide radicals. J Neurochem 2001 Mar;76(6):1895-1904.

(21) Mackenzie IR. Anti-inflammatory drugs and Alzheimer-type pathology in aging. Neurology 2000 Feb 8;54(3):732-734.

(22) Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature 2001 Nov 8;414(6860):212-216.

(23) Lim GP, Yang F, Chu T, et al. Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. J Neurosci 2000 Aug 1;20(15):5709-5714.

(24) Netland EE, Newton JL, Majocha RE, Tate BA. Indomethacin reverses the microglial response to amyloid beta-protein. Neurobiol Aging 1998 May;19(3):201-204.

(25) Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 1999 Jul 13;53(1):197-201.

(26) Aisen PS, Davis KL, Berg JD, et al. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. Neurology 2000 Feb 8;54(3):588-593.

(27) van Gool WA, Weinstein HC, Scheltens PK, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. Lancet 2001 Aug 11;358(9280):455-460.

(28) Bannwarth B, Netter P, Pourel J, Royer RJ, Gaucher A. Clinical pharmacokinetics of non-steroidal anti-inflammatory drugs in the cerebrospinal fluid. Biomed Pharmacother 1989;43(2):121-126.

Chapter 2.2

No effect of one-year treatment with indomethacin on Alzheimer's disease progression: A randomized controlled trial

D. de Jong
R.W.M.M. Jansen
W.H.L. Hoefnagels
M.J.H. Jellesma-Eggenkamp
M.M. Verbeek
G.F. Borm
H.P.H. Kremer

PLoS ONE 2008;3(1):e1475

Abstract

Objectives: To determine whether treatment with the nonselective nonsteroidal anti-inflammatory drug (NSAID) indomethacin slows cognitive decline in patients with Alzheimer's disease (AD).

Design: A double-blind, randomized, placebo-controlled trial.

Setting: The study was conducted between May 2000 to September 2005 in two hospitals in the Netherlands.

Participants: 51 patients with mild to moderate AD were enrolled into the study.

Interventions: Patients received 100 mg indomethacin or placebo daily for 12 months. Additionally, all patients received omeprazole.

Outcome measures: The primary outcome measure was the change from baseline after one year of treatment on the cognitive subscale of the AD Assessment Scale (ADAS-cog). Secondary outcome measures included the Mini-Mental State Examination, the Clinician's Interview Based Impression of Change with caregiver input, the noncognitive subscale of the ADAS, the Neuropsychiatric Inventory, and the Interview for Deterioration in Daily life in Dementia.

Results: Considerable recruitment problems of participants were encountered, leading to an underpowered study. In the placebo group, 19 out of 25 patients completed the study, and 19 out of 26 patients in the indomethacin group. The deterioration on the ADAS-cog was less in the indomethacin group (7.8 ± 7.6), than in the placebo group (9.3 ± 10.0). This difference (1.5 points; CI –4.5 - 7.5) was not statistically significant, and neither were any of the secondary outcome measures.

Conclusion: The results of this study are inconclusive with respect to the hypothesis that indomethacin slows the progression of AD.

Trial Registration: NCT00432081.

Introduction

Early indications that inflammation plays an important role in the pathogenesis of Alzheimer's disease (AD) emerged in 1982, when complement factors were found in senile plaques.(1) Many studies followed that supported the inflammatory hypothesis, and evidence accumulated that anti-inflammatory drugs, in particular nonsteroidal anti-inflammatory drugs (NSAIDs) would either prevent, postpone or treat AD.(2) However, 25 years later, there is still no clinical evidence that NSAIDs have an effect in AD patients, nor is there incontrovertible evidence of the contrary.

In a small randomized controlled trial, the traditional NSAID indomethacin appeared to protect AD patients from cognitive decline.(3) Another small randomized controlled trial studying the effect of diclofenac/misoprostol in AD, found a non-significant trend of more advanced deterioration in the placebo group than in the diclofenac/misoprostol group.(4) A large randomized controlled trial with naproxen (440 mg/d) could not confirm the earlier observed trends.(5) Both pilot studies were hampered by high withdrawal rates in the treatment groups due to side effects. Low-dose naproxen was reasonably well tolerated.

The side effects of NSAIDs, e.g. gastrointestinal toxicity, have always been a major concern that limited their use. It was suggested that the beneficial actions of NSAIDs are linked to their ability to inhibit cyclooxygenase-2 (COX-2), while their side effects result from inhibition of COX-1.(6) However, randomized controlled trials with COX-2 selective NSAIDs (rofecoxib, nimesulide, and celecoxib) failed to show an effect on the progression of AD.(5;7-9) Consequently, the traditional nonselective NSAIDs regained interest.

Apart from the promising, but never replicated, results of the initial indomethacin trial, there are also in vitro and animal model studies that support a possible therapeutic effect. Indomethacin inhibited amyloid β (Aβ)-induced neurotoxicity,(10-12) and decreased the production of Aβ-protein, interleukin-6, interleukin-1, nitric oxide, and prostaglandin E2 in a variety of cultured cells.(13-18) Furthermore, indomethacin was found to have anti-amyloidogenic effects in vitro; The formation of Aβ fibrils was dose-dependently inhibited by indomethacin.(19) In rats, indomethacin attenuated microglial infiltration, and improved lipopolysaccharide-induced amnesia.(20;21) In a transgenic mouse-model of AD-like amyloidosis (Tg2576), indomethacin

suppressed brain levels of prostaglandins,(22) and reduced Aβ levels in cortex and hippocampus.(22;23) This amyloid burden lowering effect was confirmed by other investigators using a combination of indomethacin and vitamin E to treat Tg2576 mice.(24)

Supported by these data, particularly by the prior trial that suggested a therapeutic benefit as well as by its potential Aβ lowering effect, we hypothesized that indomethacin may retard the clinical progression of AD.

Methods

Participants

Patients were recruited from May 2000 to August 2004 at the Department of Neurology and at the Memory Clinic, Department of Geriatric Medicine of the Radboud University Nijmegen Medical Center, and at the Memory clinic of the Department of Geriatric Medicine, Rijnstate Hospital, Arnhem, The Netherlands. Patients were eligible if they met the NINCDS/ADRDA criteria for the clinical diagnosis of probable AD,(25) had mild or moderate dementia as measured by a Mini-Mental State Examination (MMSE)(26) score between 10 and 26 inclusive, and were living at home or in a home for the elderly. Patients had to be supported by a reliable caregiver, who accompanied them to each clinic visit in order to provide information about the patient's functional status, and who would ensure that the participants took their test medication.

Patients were excluded if they had a history or current evidence of peptic ulceration; history of gastric surgery or gastrointestinal bleeding; severe and unstable cardiovascular disease; severe pulmonary disease; renal failure (serum creatinine greater than 200 mmol/l); clinically significant liver disease (plasma aspartate and alanine aminotransferase levels three times the upper limit of normal); poorly controlled diabetes mellitus; hypersensitivity to NSAIDs or aspirin; alcohol abuse; or advanced, severe and unstable disease of any type (other than AD), that might interfere with evaluations during the study, including a medical condition which should be expected to progress, recur, or change to such an extent that it might bias the assessment of the clinical or mental status of the patient, or put the patient

at special risk. Also, patients taking the following concomitant medications were excluded, because of a possible interaction with indomethacin; aspirin, coumarin derivatives, angiotensin converting enzyme inhibitors, loop diuretics, and long-term use of other NSAIDs or corticosteroids (more than two months immediately before study entry). Intake of the following medication was not allowed during the study because of a possible effect on cognition; estrogen replacement therapy, deprenyl, vitamin E, neuroleptics and anticholinergic medication. Patients using stable doses of cholinesterase inhibitors were eligible, with the provision that the dose should not be changed during the study. Cholinesterase inhibitors could not be initiated during the study.

At both study sites, approval of the local institutional review board to perform the study was received. Informed consent was obtained from each patient and their legally acceptable representative.

Interventions

The study was a one-year, randomized, double-blinded, placebo-controlled bicenter trial. After screening, patients were randomly assigned to receive 50 mg indomethacin twice daily or placebo twice daily for one year. In addition, patients in both treatment groups received omeprazole 20 mg once daily, to prevent gastrointestinal side effects.

Objectives

We tested whether indomethacin would have an effect on cognitive and behavioral dysfunction, as well as dysfunction of the activities of daily living, in patients with mild to moderate AD.

Outcomes

Efficacy was primarily assessed by the cognitive subscale of the AD Assessment Scale (ADAS-cog),(27) an instrument that evaluates memory, language, attention, reasoning, orientation, and praxis (range 0 to 70). Secondary outcome measures included the MMSE,(26) the Clinician's Interview Based Impression of Change with caregiver input (CIBIC+),(28) the noncognitive subscale of the ADAS (ADAS-noncog),(27) the Neuropsychiatric Inventory (NPI),(29;30) including the NPI caregiver distress scale

(NPI-D),(31) and the Interview for Deterioration in Daily life in Dementia (IDDD).(32) The IDDD is a caregiver-based measure, which consists of 20 concretely worded items that reflect the initiative to perform, and the actual performance of self-care and more complex activities.

Cognitive and behavioral assessments were performed at baseline, and at weeks 26 and 52. Safety assessments included vital signs and the recording and rating of any adverse event by the investigator (weeks 4, 8, 12, 26, 38, and 52), physical examination (baseline, week 26, and 52), and routine hematology and chemistry blood tests (baseline, week 4, 8, 26, and 52).

Sample size

The primary hypothesis tested was that indomethacin would be superior to placebo in retarding cognitive decline as measured on the ADAS-cog after one year of treatment. We aimed at 80% power to detect a 3-point difference in the change in ADAS-cog score after one year between patients who received indomethacin and those who received placebo. ADAS-cog data from previous studies were used in the power calculations for the initial trial, and an SD of 7 was assumed. This yielded a estimated sample size of 67 to be evaluated per group. Since an overall dropout rate of 20% was anticipated, the required sample size was 80 patients per group.

Randomization - Sequence Generation

The statistician provided computer-generated lists of random numbers allocating patients in a 1:1 ratio to receive indomethacin or placebo. For each center, a separate randomization list was provided.

Randomization - Allocation Concealment

Randomization codes were held by the pharmacy of the Radboud University Nijmegen Medical Center that labeled and dispensed all trial medication. Allocation was concealed from all investigators and patients.

Randomization - Implementation

Eligible patients were allocated to a randomization number in the same order they were enrolled in the trial at both trial sites. At each visit, patients received a supply of medication (indomethacin or placebo) by the pharmacy, labeled with their randomization number.

Blinding

The indomethacin and placebo tablets were of identical appearance. Neither the patients nor the investigators knew which treatment they received or dispensed. The blinding process remained complete until all data was entered in the trial database and the accuracy of the data and the database was confirmed. Afterward, the database was forwarded to the statistician for analysis.

Statistical methods

The changes from baseline in the groups were compared using analysis of covariance with the baseline results of each assessment as a covariate. In an additional analysis, gender and age were added as covariates. Two-sided p values and 95% confidence intervals were calculated. The primary efficacy analysis was conducted on the observed values. In addition, the last observation carried forward (LOCF) approach was used.

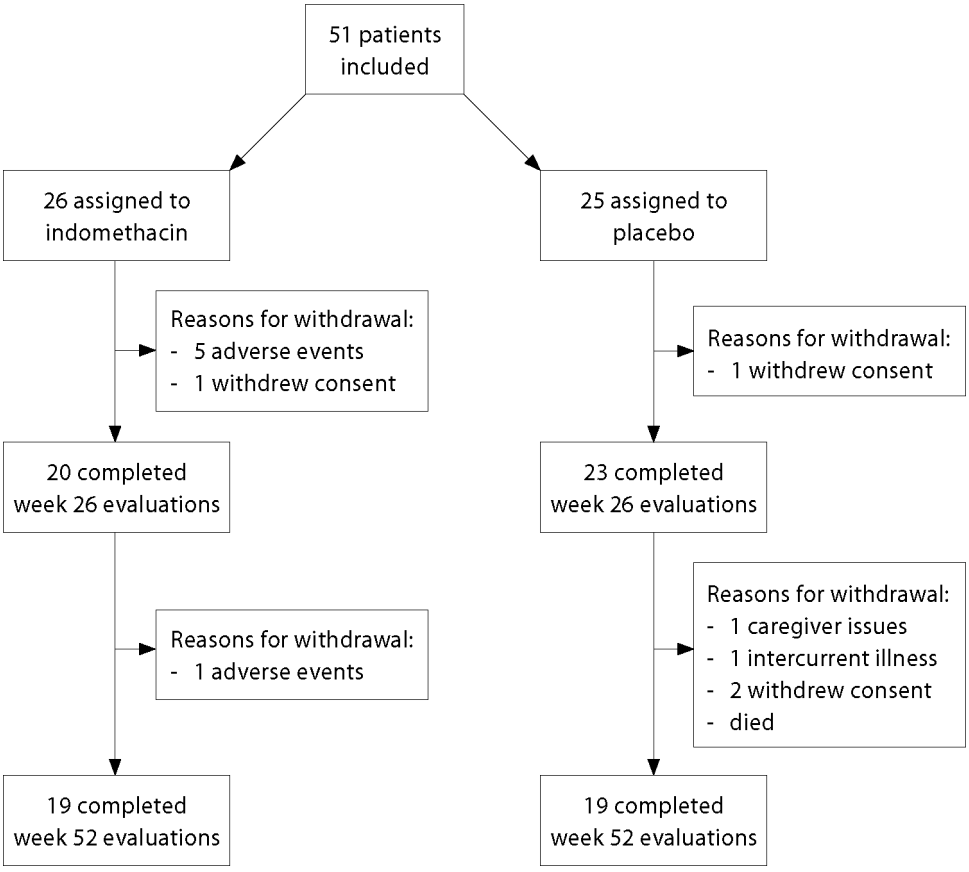
Results

Participant flow and recruitment

Figure 1 illustrates the flow of patients through the study protocol. The study was discontinued prematurely after four years, due to difficulties with the enrollment

Figure 1

Trial profile



of patients into the study. Based on an inclusion rate of approximately thirteen patients per year, eight more years of enrollment would have been necessary to complete this study. Taking into account scientific, organizational, and financial reasons, the decision was made to discontinue the study. Eventually, fifty-one patients were included in the trial, about one-thirds of the number originally anticipated. Most patients were enrolled at the Memory Clinic, Department of Geriatric Medicine of the Radboud University Nijmegen Medical Center (n = 46), with an inclusion rate of one out of every five to six patients diagnosed with AD. The remainder of patients was enrolled at the outpatient clinic of the Department of Neurology of the Radboud University Nijmegen Medical Center (n = 3), and at the Department of Geriatric Medicine of the Rijnstate Hospital, Arnhem (n = 2).

Numbers analyzed

Twenty-five patients were randomly assigned to the placebo group, and twenty-six patients to the indomethacin group. Completion rates were 19 of 25 patients (76%) in the placebo group, and 19 of 26 patients (73%) in the indomethacin group. One patient in the indomethacin group discontinued the study in week 48 due to caregiver issues, but completed all week 52 evaluations. The predominant reasons for premature study discontinuation were adverse events (n = 6) in the indomethacin group, and withdrawal of consent (n = 2) in the placebo group. None of the patients that withdrew from the study due to adverse events did complete their follow-up assessments, however all other available assessment data were included in the analysis.

Baseline data

Treatment groups were similar with respect to demographic and baseline clinical characteristics, except for gender distribution (table 1); in the placebo group 24% of patients were male, and in the indomethacin group 46% of patients. No significant differences were found between baseline assessment scores. Nevertheless, baseline NPI, NPI-D, and ADAS-noncog scores were higher in the indomethacin group, suggesting that patients in this group had more behavioral problems.

Table 1

Baseline characteristics of the study population by treatment group

Characteristics	Placebo (n = 25)	Indomethacin (n = 26)
Men / women	6/19	12/14
Age (SD), years	72.2 (9.0)	72.7 (6.9)
Education level (SD), range 1 to 5*	2.7 (0.9)	2.4 (1.3)
≥1 APOE ε4 allele, n (%)	11 (44%)	13 (50%)
Disease duration (SD), months	31.1 (19.6)	32.9 (17.4)
Use of cholinesterase inhibitor, n (%)	2 (8,0%)	2 (7.7%)
MMSE score (SD)	20.2 (3.9)	19.1 (4.1)
ADAS-cog score (SD)	19.7 (8.8)	20.2 (8.3)
ADAS-noncog score (SD)	2.8 (2.7)	3.5 (3.6)
NPI score (SD)	7.1 (6.7)	11.2 (12.0)
NPI-D score (SD)	5.6 (4.5)	7.7 (7.3)
IDDD score (SD)	21.2 (12.8)	22.8 (13.7)

* level 1 is primary school only; level 5 is university level.

Outcomes, estimation, and ancillary analyses

The effect of treatment on primary and secondary outcome measures is shown in table 2. The decrease in mean ADAS-cog score after one year of therapy was 1.5 points less in the indomethacin group (7.8 ± 7.6) compared to the placebo group (9.3 ± 10.0), however this was not statistically significant (CI –4.5 - 7.5). When using the LOCF approach to analyze the difference in change in ADAS-cog score, or when gender and age were included as covariate in the analysis, the results were similar to the primary analysis (data not shown).

The decline of secondary outcome measures after six months or one year of treatment did not show statistically significant differences between groups either (table 2). Additional analysis, using the LOCF approach, showed similar results.

Table 2

Mean change from baseline of outcome measures, and difference in scores between the placebo and indomethacin group, after six and twelve months of treatment

	Placebo group mean change from baseline (SD)		Indomethacin group mean change from baseline (SD)		Difference between groups* (95% CI)	
Measure	6 months (n = 23)	1 year (n = 19)	6 months (n = 20)	1 year (n = 19)	6 months	1 year
ADAS-cog	3.9 (4.5)	9.3 (10.0)	4.8 (5.8)	7.8 (7.6)	-0.9 (-4.1 - 2.2)	1.5 (-4.5 - 7.5)
ADAS-noncog	-0.3 (1.5)	1.6 (4.2)	1.5 (4.1)	3.8 (6.7)	-1.8 (-3.9 - 0.2)	-2.8 (-6.7 - 1.1)
MMSE	-2.4 (3.6)	-5.4 (5.5)	-2.3 (3.2)	-3.4 (4.3)	0.1 (-1.9 - 2.1)	1.6 (-1.6 - 4.8)
NPI	-0.3 (4.9)	9.4 (14.0)	1.7 (14.0)	3.2 (18.1)	-3.6 (-10.1 - 2.9)	4.6 (-6.6 - 15.8)
NPI-D	-0.9 (3.5)	6.5 (8.8)	0.7 (6.4)	1.4 (8.3)	-2.2 (-5.4 - 1.0)	4.6 (-1.3 - 10.5)
IDDD	10.4 (8.3)	18.2 (14.8)	9.5 (14.4)	19.4 (13.8)	0.8 (-6.4 - 8.0)	-1.5 (-11.0 - 8.0)
CIBIC+	5.3 (0.7)	5.7 (0.7)	5.1 (0.8)	5.6 (0.8)	0.2 (-0.2 - 0.6)	0.1 (-0.3 - 0.5)

* differences, adjusted for baseline (analysis of covariance)
Negative change in scores from baseline indicates improvement, with the exception of the MMSE score (positive change indicates improvement), and the CIBIC+ (higher score means worse compared to baseline). Positive difference between groups means in favor of the indomethacin group, for all measures.

Adverse Events

Blood test abnormalities, abnormalities found during physical examination, and adverse events reported on case report forms were grouped into categories for analysis. Adverse events that occurred in at least two patients in either treatment group are listed in table 3. Patients in the indomethacin group had more frequent adverse

events. Dyspepsia, epigastic pain, or abdominal distress or pain, were reported more frequently in the placebo group (n = 3), than in the indomethacin group (n = 1). In both groups, there were no reports of serious gastrointestinal adverse events, such as gastroenteritis, ulceration or bleeding. Nausea, dizziness, and hyperglycemia were more common in the indomethacin group, whereas diarrhea, constipation, and headache, were more common in the placebo group. Weight loss, defined as 5 percent or more loss of body weight, was seen in three patients in the indomethacin group, and in one patient in the placebo group. New cases of hypertension were reported more frequently in the indomethacin group (5 out of 22 non-hypertensive patients at baseline; 23%), than in the placebo group (2 out of 18 non-hypertensive patients at baseline; 11%). Despite these cases of elevated blood pressure, the change in mean arterial pressure (MAP) during the trial was not significantly different between groups; MAP increased 2.5 ± 10.6 (mean ± SD) mmHg in the indomethacin group, and decreased 1.2 ± 9.5 mmHg in the placebo group (p = 0.20).

Table 3

Adverse events that occurred in at least two patients in either treatment group

Adverse event	Placebo and omeprazole (n = 25)	Indomethacin and omeprazole (n = 26)
Nausea	0	2
Diarrhea or constipation	3	2
Dyspepsia, epigastric or abdominal pain	3	1
Weight loss (≥ 5% during the study)	1	3
Headache	2	0
Dizziness	1	3
Hyperglycemia	1	2
Hypertension (new cases)	2	5

Serious adverse events were also more common in the indomethacin group (n = 5) than in the placebo group (n = 1; table 4), and reason for study withdrawal (table 4). In the indomethacin group, blood tests revealed a considerable elevation of creatinine

levels (> 1.5 times the upper limit of normal) in three patients, without clinical symptoms. All three patients had abnormal creatinine clearance rates before entering the trial, and one of these patients had a history of nephrectomy. After discontinuation of the study, serum creatinine levels returned to their previous levels. Blood tests also revealed increased levels (> 3 times the upper limit of normal) of alanine aminotransferase, and aspartate aminotransferase in one patient in the indomethacin group, without clinical symptoms. Liver function tests normalized within four weeks after study discontinuation. Nine days after enrollment in the study, one patient in the indomethacin group had a lacunar stroke. Evaluation after four months of recovery revealed only minor disabilities (increased memory impairment and irritability). Death occurred in one patient in the placebo group after 38 weeks of study participation. The cause of death of this patient is unknown.

Table 4

Serious adverse events

Serious adverse event	Placebo and omeprazole (n = 25)	Indomethacin and omeprazole (n = 26)
Elevated creatinine*	0	3
Abnormal liver function tests†	0	1
Stroke (lacunar)	0	1
Death	1	0

* > 1.5 times the upper limit of normal; † > 3 times the upper limit of normal

Discussion

Interpretation

In this study, indomethacin 50 mg twice daily did not show any statistically significant effects on the progression of dementia in patients with mild to moderate AD during a 1-year period, as measured by testing of cognition, behavior, and activities of daily living, and by overall clinical global impression.

Although our study included more patients than the earlier trials with indomethacin and diclofenac/misoprostol, the number of included patients was still too small.(3;4) Thus, the study was clearly underpowered, resulting in very wide confidence intervals; The confidence interval for the ADAS-cog was 12 points (range –4.5 to 7.5). This means that the difference between the groups should have been at least 6 points to reach statistical significance.

Generalizability

The enrollment of patients was hampered by the extensive exclusion criteria, especially the exclusion of patients using aspirin, angiotensin converting enzyme inhibitors or loop diuretics. The institutional review board specifically imposed this criterion, since interaction of these drugs with indomethacin might aggravate the occurrence of side effects of indomethacin. Not only did patient enrollment suffer from these strict criteria, it is also responsible for another limitation of the study; Our study population was a highly selected group of AD patients, with no or minor cardiovascular comorbidity, and thus not representative of the average AD population.

Overall evidence

By its nature our study cannot prove that anti-inflammatory drugs in general and indomethacin in particular are ineffective. However, the study outcome is consistent with earlier trials that investigated prednisone, hydroxychloroquine, and various selective and non-selective NSAIDs in similar designs; All these studies failed to demonstrate a beneficial effect on disease progression.(4;5;7-9;33;34) These failures may have been due to the pharmacokinetic or pharmacological properties of the drugs being used. But it may also be questioned whether anti-inflammatory treatment will ever be efficacious in treating symptomatic AD. Although they may have preventive effects, they may no longer be effective in patients with established disease.

Indomethacin in combination with omeprazole was reasonably well tolerated in this elderly population. There were no serious gastrointestinal tract events. Dyspepsia, epigastic pain, or abdominal distress or pain were more common in the placebo group, and may have been caused by omeprazole, and not by indomethacin. However, elderly patients should be carefully monitored when using indomethacin. Blood pressure should be checked regularly, and blood tests must be done before

and during indomethacin treatment. In patients with elevated creatinine clearance, the administration of indomethacin should be avoided.

In conclusion, the results of this study are inconclusive with respect to the hypothesis that indomethacin slows the progression of AD. Owing to its limited statistical power, this study does not alter the conclusions from earlier trials that NSAIDs do not appear to be effective in altering the progression of symptoms in AD. Thus, treatment of AD patients with indomethacin should currently not be recommended, and further treatment trials with NSAIDs in AD patients should be thoroughly reconsidered. However, primary prevention trials with NSAIDs, in particular ibuprofen (in combination with omeprazole), are warranted to further investigate the effect of long-term NSAID use on risk of AD.

References

(1) Eikelenboom P, Stam FC. Immunoglobulins and complement factors in senile plaques. An immunoperoxidase study. Acta Neuropathol Berl 1982;57(2-3):239-242.

(2) McGeer PL, McGeer EG. NSAIDs and Alzheimer disease: Epidemiological, animal model and clinical studies. Neurobiol Aging 2006 May 10;28(5):639-647.

(3) Rogers J, Kirby LC, Hempelman SR, et al. Clinical trial of indomethacin in Alzheimer's disease. Neurology 1993;43(8):1609-1611.

(4) Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 1999 Jul 13;53(1):197-201.

(5) Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. JAMA 2003 Jun 4;289(21):2819-2826.

(6) Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998;38:97-120.

(7) Reines SA, Block GA, Morris JC, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 2004 Jan 13;62(1):66-71.

(8) Aisen PS, Schmeidler J, Pasinetti GM. Randomized pilot study of nimesulide treatment in Alzheimer's disease. Neurology 2002 Apr 9;58(7):1050-1054.

(9) Sainati SM, Ingram DM, Talwalker S, Geis GS. Results of a double-blind placebo-controlled study of celecoxib for the progression of Alzheimer's disease. Proceedings of the sixth International Stockholm-Springfield Symposium of advances in Alzheimer therapy 2000;p.180.

(10) Dzenko KA, Weltzien RB, Pachter JS. Suppression of A beta-induced monocyte neurotoxicity by antiinflammatory compounds. J Neuroimmunol 1997;80(1-2):6-12.
(11) Fagarasan MO, Efthimiopoulos S. Mechanism of amyloid beta-peptide (1-42) toxicity in PC12 cells. Mol Psychiatry 1996 Nov;1(5):398-403.
(12) Fagarasan MO, Aisen PS. IL-1 and anti-inflammatory drugs modulate A beta cytotoxicity in PC12 cells. Brain Res 1996;723(1-2):231-234.
(13) Weggen S, Eriksen JL, Das P, et al. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature 2001 Nov 8;414(6860):212-216.
(14) Kadoyama K, Takahashi Y, Higashida H, Tanabe T, Yoshimoto T. Cyclooxygenase-2 stimulates production of amyloid beta-peptide in neuroblastoma x glioma hybrid NG108-15 cells. Biochem Biophys Res Commun 2001 Feb 23;281(2):483-490.
(15) Blom MA, van Twillert MG, de Vries SC, et al. NSAIDS inhibit the IL-1 beta-induced IL-6 release from human post-mortem astrocytes: the involvement of prostaglandin E2. Brain Res 1997;777(1-2):210-218.
(16) Bour AM, Westendorp RG, Laterveer JC, Bollen EL, Remarque EJ. Interaction of indomethacin with cytokine production in whole blood. Potential mechanism for a brain-protective effect. Exp Gerontol 2000 Oct;35(8):1017-1024.
(17) Hoozemans JJ, Veerhuis R, Janssen I, Rozemuller AJ, Eikelenboom P. Interleukin-1beta induced cyclooxygenase 2 expression and prostaglandin E2 secretion by human neuroblastoma cells: implications for Alzheimer's disease. Exp Gerontol 2001 Mar;36(3):559-570.
(18) Du ZY, Li XY. Inhibitory effects of indomethacin on interleukin-1 and nitric oxide production in rat microglia in vitro. Int J Immunopharmacol 1999 Mar;21(3):219-225.
(19) Hirohata M, Ono K, Naiki H, Yamada M. Non-steroidal anti-inflammatory drugs have anti-amyloidogenic effects for Alzheimer's beta-amyloid fibrils in vitro. Neuropharmacology 2005 Dec;49(7):1088-1099.
(20) Netland EE, Newton JL, Majocha RE, Tate BA. Indomethacin reverses the microglial response to amyloid beta-protein. Neurobiol Aging 1998 May;19(3):201-204.
(21) Ma TC, Zhu XZ. Suppression of lipopolysaccharide-induced impairment of active avoidance and interleukin-6-induced increase of prostaglandin E2 release in rats by indometacin. Arzneimittelforschung 1997;47(5):595-597.
(22) Sung S, Yang H, Uryu K, et al. Modulation of nuclear factor-kappa B activity by indomethacin influences A beta levels but not A beta precursor protein metabolism in a model of Alzheimer's disease. Am J Pathol 2004 Dec;165(6):2197-2206.
(23) Quinn J, Montine T, Morrow J, Woodward WR, Kulhanek D, Eckenstein F. Inflammation and cerebral amyloidosis are disconnected in an animal model of Alzheimer's disease. J Neuroimmunol 2003 Apr;137(1-2):32-41.

(24) Yao Y, Chinnici C, Tang H, Trojanowski JQ, Lee VM, Pratico D. Brain inflammation and oxidative stress in a transgenic mouse model of Alzheimer-like brain amyloidosis. J Neuroinflammation 2004 Oct 22;1(1):21.

(25) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984 Jul;34(7):939-944.

(26) Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975 Nov;12(3):189-198.

(27) Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. Am J Psychiatry 1984 Nov;141(11):1356-1364.

(28) Knopman DS, Knapp MJ, Gracon SI, Davis CS. The Clinician Interview-Based Impression (CIBI): a clinician's global change rating scale in Alzheimer's disease. Neurology 1994 Dec;44(12):2315-2321.

(29) Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. Neurology 1994 Dec;44(12):2308-2314.

(30) Kat MG, de Jonghe JF, Aalten P, Kalisvaart CJ, Droes RM, Verhey FR. Neuropsychiatric symptoms of dementia: psychometric aspects of the Dutch Neuropsychiatric Inventory (NPI). Tijdschr Gerontol Geriatr 2002 Sep;33(4):150-155.

(31) Kaufer DI, Cummings JL, Christine D, et al. Assessing the impact of neuropsychiatric symptoms in Alzheimer's disease: the Neuropsychiatric Inventory Caregiver Distress Scale. J Am Geriatr Soc 1998 Feb;46(2):210-215.

(32) Teunisse S, Derix MM. The interview for deterioration in daily living activities in dementia: agreement between primary and secondary caregivers. Int Psychogeriatr 1997;9 Suppl 1:155-162.

(33) Aisen PS, Davis KL, Berg JD, et al. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. Neurology 2000 Feb 8;54(3):588-593.

(34) van Gool WA, Weinstein HC, Scheltens PK, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. Lancet 2001 Aug 11;358(9280):455-460.

Chapter 2.3

External validity of a randomized controlled trial in Alzheimer's disease

D. de Jong
H.P.H. Kremer
M.M. Verbeek
G.F. Borm
R.W.M.M. Jansen

Submitted

Abstract

Objectives: To investigate the external validity of the results of a randomized controlled trial (RCT) with indomethacin in Alzheimer's disease (AD) patients, and evaluate the generalizability of other drug-trials in AD patients.

Design: Retrospective study / review.

Setting: Memory clinic.

Participants: All AD patients that participated in a RCT with indomethacin (RCT group, n = 51), versus all remaining AD patients seen at the memory clinic for diagnosis and treatment during the four-year recruitment period of the trial (control group, n = 128).

Measurements: Characteristics of patients, such as medication use, comorbidity, results of physical and neurological examination, were collected. The Cumulative Illness Rating Scale for geriatrics (CIRS-G) was used to assess the presence and severity of comorbidity. Furthermore, 72 RCTs with AD patients testing various drugs were selected from the literature for further comparisons.

Results: Age of the RCT group was significantly lower (72.4 ± 8.0 years) compared to the control group (76.1 ± 6.5 years; $p<0.01$). Furthermore, the RCT group had fewer disabilities and comorbid conditions, and were taking less medication, than the control group. In 62 out of 72 evaluated other RCTs, mean age of participating patients was < 76 years. In only 11 RCT articles, some information was available on medication use or comorbidity of participants.

Conclusion: The external validity of the results of our RCT with indomethacin and of many other RCTs with AD patients is limited. Care should be taken to extrapolate conclusions from clinical trials to a general population of AD patients.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that afflicts millions of people worldwide, and this number is expected to increase significantly in the coming decades. Since 1990, many randomized controlled trials (RCTs) have been conducted in AD patients testing different types of drugs. RCTs are considered the gold standard for determining the efficacy and safety of treatment. To be clinically useful, the results of RCTs should be relevant to patients in routine clinical practice, i.e. the external validity, generalizability or applicability should be high.(1;2) The external validity of RCTs, however, may be limited by stringent protocol-driven conditions, such as restrictions in disease severity, co-morbid conditions, and concomitant medications, as well as limited follow-up periods and sample sizes. These conditions, and many others, have to be taken into account when interpreting the results of an RCT. More specifically, physicians should consider if patients in their care might respond differently to treatment than patients studied in a clinical trial.

Despite the fact that numerous drug-treatment trials have been conducted in AD, there are only a few reports reviewing the quality of these RCTs. The external validity of an RCT with the cholinesterase inhibitor tacrine has been discussed, in which an enrichment strategy was used.(1;3;4) In this study, 632 AD patients were first enrolled in a run-in phase, in which their responsiveness to tacrine and best dose was determined. After a washout period, only patients who had an improvement on tacrine (n = 215) were randomly assigned to receive either placebo or their best dose of tacrine in the double-blind main phase of the trial. This approach undermines the generalizability of the results of this trial. Nevertheless, other RCTs were conducted using the same controversial strategy.(5-7)

Another study discussed the high selection of AD patients recruited to drug trials.(8) The authors investigated the records of 279 AD patients seen in their clinic who were all candidates for drug trials. From these patients, only 36 patients were enrolled in a clinical trial (13%). There were various reasons for non-enrollment, for example the occurrence of behavioral symptoms that required treatment (n = 61), and concomitant diseases (n = 30). Enrollment was more likely to occur in patients with higher Mini-Mental State Examination (MMSE) scores,(9) higher schooling level, and younger age. Other investigators found that AD patients, who provisionally fulfilled

the selection criteria of two different clinical trials, were better educated, wealthier, more likely to be white, younger, and relatively underrepresented by women.(10) Furthermore, they found that more than 60% of patients were excluded because of significant behavioral problems, and approximately one-quarter because of significant medical or neurological problems. The age-gap between dementia patients in clinical studies and dementia patients in the general population, was also addressed. (11) These investigators concluded that dementia research is systematically biased towards patients who are relatively young compared to the general dementia population.

Discussing external validity of AD drug trials raises many questions. Are AD patients who are mainly recruited in research centers comparable to patients from a community-based outpatient clinic or memory clinic? Do common eligibility and exclusion criteria lead to a selection bias in groups of AD patients? Is the general health of patients included in RCTs similar to that of patients in routine daily practice? Since most published trial reports list only a few baseline characteristics (usually age, race, disease duration, and MMSE score), these questions cannot be answered. It should be noted that the importance of measuring the presence and severity of comorbidity in effectiveness studies in AD was already addressed in 1997.(12)

Based on the observations described above, we hypothesize that AD patients participating in RCTs evaluating the effect of different drugs are not representative of AD patients seen in routine daily practice. In order to address this, we studied the characteristics of the patients who visited our memory clinic, and compared them to the characteristics of patients we had enrolled in a RCT conducted by ourselves,(13) as well as to patients who were enrolled in published trials. Our formal aim was to analyze how our inclusion and exclusion criteria potentially affected the validity of a trial like the one we published.

Methods

Patients

AD patients that entered the control group were selected from patients referred by family physicians or other medical specialists to the memory clinic of the Radboud University Nijmegen Medical Center, between April 2000 and April 2004 (a total of 420 patients). In the Netherlands, AD patients eligible for treatment are diagnosed and treated at memory clinics, or alternatively at an outpatient clinic of a Geriatric Medicine or Neurology Department, especially since prescription of cholinesterase inhibitors is restricted to geriatricians and neurologists. There is only one memory clinic in the region of Nijmegen, thus the AD population seen at our clinic may be a good representation of AD patients from the general population eligible for drug-treatment. All patients followed an extensive diagnostic examination protocol. The final diagnosis was based on the NINCDS/ADRDA criteria,(14) and was established by a panel of geriatricians and a neurologist specialized in dementia. All patients diagnosed with probable AD that were not participating in the RCT with indomethacin, were included in the control group (n = 128).

The RCT group consisted of AD patients that participated in our previously described RCT with the nonsteroidal anti-inflammatory drug (NSAID) indomethacin (n = 51).(13) During a four-year period (2000 to 2004), patients were recruited at the memory clinic of the Department of Geriatric Medicine (n = 46), and at the outpatient clinic of the Department of Neurology (n = 3) of the Radboud University Nijmegen Medical Center, as well as at the memory clinic of the Department of Geriatric Medicine, Rijnstate Hospital, Arnhem, the Netherlands (n = 2). Patients were eligible if they met the NINCDS/ADRDA criteria for the clinical diagnosis of probable AD,(14) had mild or moderate dementia as measured by a MMSE score between 10 and 26 inclusive, were living at home or in a home for the elderly, and were supported by a reliable caregiver. Patients were excluded if they had a history or current evidence of peptic ulceration; history of gastric surgery or gastrointestinal bleeding; severe and unstable cardiovascular disease; severe pulmonary disease; renal failure (serum creatinine > 200 mmol/l); clinically significant liver disease (plasma aspartate and alanine aminotransferase levels three times the upper limit of normal); poorly controlled diabetes mellitus; hypersensitivity to NSAIDs or aspirin; alcohol abuse; or advanced,

severe and unstable disease of any type (other than AD), that might interfere with evaluations during the study, or put the patient at special risk. Also, patients taking the following concomitant medications were excluded, because of a possible interaction with indomethacin; aspirin, coumarin derivatives, angiotensin converting enzyme (ACE) inhibitors, loop diuretics, corticosteroids, and other NSAIDs. Intake of the following medication was not allowed during the study because of a possible effect on cognition; estrogen replacement therapy, deprenyl, vitamin E, neuroleptics, and anticholinergic medication. It should be emphasized that subjects not included in the RCT were included in the control group.

Data collection

Data on duration of the disease, severity of cognitive impairment, medical history, medication, intoxications, level of education, functional impairment, gait disorders, physical and neurological examination, neuropsychological testing, laboratory testing, and imaging of the brain were extracted from the medical charts.

Outcome measures

For the current analysis the MMSE score was included,(9) since this rapid neuropsychological test was assessed in almost every patient (control group, n = 120 (94%); RCT group, n = 51 (100%)). Severity of dementia was rated using the Clinical Dementia Rating scale.(15) Duration of the disease was defined as time in years between first symptoms and diagnosis. Level of education was rated on a scale of 1 (primary school level) to 5 (university level). The level of dependence of patients on their caregivers regarding the activities of daily living (ADL) and instrumental activities of daily living (IADL) was scored as follows: (0) independent, (1) partially dependent, (2) dependent. Height and weight were used to calculate the Body Mass Index (BMI). Mean arterial pressure (MAP) of patients was calculated using values of systolic and diastolic blood pressure. Plasma concentrations of sodium, potassium, creatinine, glucose, haemoglobin, and TSH were recorded, and out of range plasma concentrations were registered. Laboratory results of a patient were scored abnormal if one or more test results were out of range.

The Cumulative Illness Rating Scale for geriatrics (CIRS-G) was used to assess comorbidity.(16;17) This rating scale scores the presence and severity of comorbid

disease in 14 organ-specific categories (heart; vascular; haematopoietic; respiratory; eyes, ears, nose and throat and larynx; upper gastrointestinal tract; lower gastrointestinal tract; liver; renal; genitourinary tract; musculoskeletal/integument; neurological; endocrine/metabolic and breast; and psychiatric illness). The worst problem in each category is rated on a scale from 0 to 4 (0 = no comorbidity; 4 = extremely severe/immediate treatment required/end-organ failure/severe impairment in function). We excluded category 14 (psychiatric illness), since this item scores the presence and severity of dementia. Two scores were calculated: (1) the total CIRS-G score, which is the sum score of all 13 organ-specific categories, and (2) the CIRS-G severity index, which is the ratio of the total score and the number of endorsed categories.

Polypharmacy was defined as use of five or more drugs.(18) Only prescription drugs that were taken on a regular basis (not as-needed) were included. All drugs were categorized in different drug classes to enable subgroup analysis.

Statistical analysis

The two-sample t- test and the chi-squared test were used for group comparisons. In addition, analysis of covariance and logistic regression were carried out, with covariate age. Pearson's correlation coefficient was used for analyzing correlations. Results with two-sided p-value less than 0.05 were considered statistically significant.

Comparison with published trials

MEDLINE and PubMed (1990 to March 2009) were systematically searched using the terms Alzheimer's disease, and randomized clinical trial. All articles were reviewed using the following predetermined inclusion criteria: (1) the trial was randomized, double-blinded, and placebo-controlled; (2) patients enrolled were diagnosed as having mild to moderate AD; (3) thirty or more participating patients; (4) the trial involved 12 or more weeks of continuous drug treatment; (5) the effect on cognitive function was measured with the cognitive subscale of the Alzheimer Disease Assessment Scale (ADAS-cog); and (6) data were available about age, and selection criteria of patients. After identification of all relevant trials, we extracted the following information; diagnostic criteria used for AD, inclusion criteria (MMSE score range, age range), exclusion criteria, trial duration, number of participating sites, mean age of participating patients, and availability of baseline characteristics of patient groups

(gender, race, level of education, MMSE score, duration of the disease, weight, concomitant diseases, and concomitant medication). In addition, we calculated mean age, and mean MMSE score of participating patients.

Table 1

Clinical characteristics of Alzheimer's disease patients in the control group and the randomized controlled trial group

	Control group (n = 128)	RCT group (n = 51)	p-value
Age, years	76.1 ± 6.5	72.4 ± 8.0	< 0.01
Sex (male/female), %	34/66	35/65	0.91
MMSE score	18.2 ± 5.5	19.7 ± 4.0	0.24
Clinical Dementia Rating scale	1.4 ± 0.6	1.3 ± 0.6	0.35
Disease duration, years	2.5 ± 2.0	2.7 ± 1.5	0.15
Level of education[#]	2.3 ± 1.3	2.5 ± 1.1	0.22
Smoking, n (%)	23 (21)	10 (20)	0.85
Alcohol, units/week	4.1 ± 6.9	4.5 ± 7.1	0.75
ADL dependence score[†]	0.4 ± 0.7	0.1 ± 0.3	< 0.01
IADL dependence score[†]	1.2 ± 0.7	1.0 ± 0.5	< 0.05
Gait disorder, n (%)	18 (14)	5 (10)	0.46
Body mass index	25.6 ± 3.9	25.4 ± 3.2	0.97
Mean arterial pressure, mm Hg	108 ± 16	106 ± 13	0.44
Laboratory results abnormal*, n (%)	67 (53)	20 (42)	0.19
CIRS-G total score	6.7 ± 3.9	5.3 ± 2.8	< 0.05
CIRS-G severity index	1.8 ± 0.6	1.5 ± 0.4	< 0.001
No. of drugs used	2.7 ± 2.5	1.4 ± 1.6	< 0.01
Polypharmacy (≥ 5 drugs), n (%)	31 (24)	3 (6)	< 0.01

[#]level 1 is primary school only; level 5 is university level; * ≥ 1 test result abnormal: sodium, potassium, creatinine, glucose, haemoglobin, and TSH; [†] score: 0 = independent, 1 = partially dependent, 2 = dependent
Abbreviations: MMSE = Mini-Mental State Examination; ADL = activities of daily living; IADL = instrumental activities of daily living; CIRS-G = Cumulative Illness Rating Scale for geriatrics.

Results

As listed in Table 1, age was significantly lower in the RCT group than in the control group. Gender distribution, MMSE score and the Clinical Dementia Rating scale score were similar in both groups. No significantly differences in disease duration, level of education, smoking, and alcohol intake were found between groups. In the control group, patients were more dependent on their caregivers for ADL and IADL, than patients in the RCT group. In both groups, there was a similar percentage of patients with gait disorders.

Physical examination revealed no differences in BMI or mean arterial pressure (MAP) between groups. In both groups, there was a similar percentage of patients with one or more abnormal laboratory tests. However, glucose levels in the control group were significantly higher than in the RCT group (5.7 ± 2.0 mmol/l vs. 4.9 ± 0.7 mmol/l; $p < 0.05$). All other laboratory results were similar in both groups.

The CIRS-G total score and CIRS-G severity index were significantly higher in the control group compared to the RCT group (Table 1). Also, a moderate positive correlation between the CIRS-G total score and age ($r = 0.39$; $p < 0.001$) was found. Furthermore, when both differences in CIRS-G total score and the CIRS-G severity index between groups were corrected for age, they were still statistically significantly (both $p < 0.05$). When analyzing the individual organ-specific categories of the CIRS-G, we found significant differences between groups in the categories "Heart" (control group 0.9 ± 1.1; RCT group 0.2 ± 0.5; $p < 0.001$), and "Endocrine/metabolic and breast" (control group 0.5 ± 0.8; RCT group 0.2 ± 0.5; $p < 0.05$). There were no significant differences between groups in the other categories.

In the control group, patients were using significantly more drugs than in the RCT group (Table 1). The number of prescribed drugs was positively correlated with the CIRS-G total score ($r = 0.57$; $p < 0.001$). Furthermore, polypharmacy was much more common in the control group than in the RCT group. Analysis of the different drug classes revealed a significantly more frequent use in the control group versus the RCT group of β-blockers (28% vs. 17 %; $p < 0.05$), and digoxin (9% vs. 0%; $p < 0.05$). In the control group 19% of patients were using ACE inhibitors, 12% coumarins, and 19% antiplatelet agents, compared to 0% in the RCT group, since patients using these drugs were excluded from the trial.

Our systematic literature search resulted in 72 RCTs (see appendix for all references) with AD patients testing various drugs. Nine out of 72 RCTs were multi-center trials, with a minimum of 2 participating sites, and a maximum of 100 sites. Most RCTs included only patients with probable AD according to the NINCDS-ADRDA criteria, however five trials also included patients with possible AD,(19-23) and two trials included patients with AD according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV) criteria.(24;25) Reviewing the inclusion criteria of different trials, we found different age ranges; Twenty-one out of 72 trials included all patients older than 50 years of age. In other trials, minimum age varied from 40 (21;26-29) to 65 years of age,(30;31) and maximum age varied from 80 (30;31) to 95 years of age.(32) Also, in 16 RCTs, there was no age range at all. Although all trials included patients with 'mild to moderate AD', the lower limit of the MMSE score varied from 8 (33) to 16 points,(34) and the upper limit varied from 20 (35) to 30 points.(36) Two trials only included patients in good or excellent general health.(37;38) More frequently, comorbid conditions were mentioned in the exclusion criteria; In 56 out of 72 trials, patients with a 'serious unstable illness', 'clinically significant concomitant disease', 'clinically significant coexisting medical abnormality', or 'major medical illness' were excluded. Also, comedication was a frequent reason for excluding patients (35 out of 72 trials), especially patients using 'medication known to affect the central nervous system', or 'psychotropic medication'. Some trials excluded patients with specific comedication, such as cholinesterase inhibitors, or anticoagulantia.

In the majority of RCT articles, only a few baseline characteristics were reported, mostly to compare the placebo group with the group of patients receiving the study drug. Mean age of participants per group was present in all articles; Mean age of all participants of all RCTs was 73.4 (range 66,6 – 78.6) years of age. Also, mean MMSE score of participants per group was frequently available; Mean MMSE score of all participants of all RCTs was 19.4 (range 16.4 to 23.4) points. Other frequently available baseline characteristics of patients were race (36 out of 72), weight (27/72), disease duration (26/72), and level of education (24/72). In only 6/72 RCT articles, number or percentage of patients with comorbid conditions were reported.(5;39-43) In two articles, more specific comorbid conditions were presented: the percentage of patients with a cardiovascular history, and number of patients with diabetes.(36;44) Mean number of concomitant drugs per patient, or number and percentage of

patients using concomitant medication were described in some articles (8/72);(5;39-43;45;46) 6 out of 8 articles also reported number and percentage of patients taking psychotropic drugs.(5;40-42;45) Three articles only gave details about number and percentage of patients taking several specific types of medication.(20;36;46)

Discussion

We recently reported on a randomized controlled clinical trial of indomethacin versus placebo that aimed to show retardation of disease progression in AD patients. This study failed to show any effect, although conclusions could not be drawn since this trial was underpowered. In the present study, we demonstrate that our eligibility criteria resulted in a cohort of participating AD patients who were younger, had fewer disabilities and comorbid conditions, and were taking less medication, than the AD patients in general who visited our memory clinic in the same period. Apparently, through our strict eligibility criteria we have selected a group of patients for our RCT that is not representative of AD patients in our own memory clinic, most likely not representative of AD patients in the general population.

The relatively young age of AD patients participating in RCTs compared to AD patients seen in routine daily practice (mean age of our reference AD population was 76.1 years), is a common problem seen in most trials over the past seventeen years. Amongst the 72 RCTs we selected from the literature, we only found ten studies with a mean age of participating patients ≥ 76 years of age.(21;22;37;47-53) Although age in our population of AD patients was positively correlated with the CIRS-G scores and number of prescribed drugs, younger age also was an independent predictor for eligibility. Therefore, we confirm the observations of two previous studies, that enrollment in RCTs that test drugs in AD patients is more likely to occur in AD patients of younger age.(8;10)

Another finding was that in the RCT group, patients had fewer ADL and IADL disabilities than in the control group. This suggests that AD patients in the control group were functionally more impaired than patients in the RCT group, even though there were no significant differences between groups in score on the Clinical Dementia Rating scale or the MMSE.

In view of the exclusion criteria of the indomethacin trial, our findings of less polypharmacy, lower medication use, less comorbidity, and also less severe comorbidity in the RCT group were not unexpected. Since there is not many information available in other RCT articles about comorbidity, one can only speculate about the number and severity of comorbid diseases of participating AD patients. When evaluating the eligibility criteria of the selected RCTs, we found that subjects were in- or excluded from trials using unclear descriptions concerning comorbidity, such as 'good to excellent general health', and 'clinically significant medical conditions'. Some studies provided more detailed descriptions about the concomitant diseases that were reason for non-enrollment. Thus, this might suggest that eligible AD patients in other RCTs have less comorbidity, as well as less severe comorbidity. In accordance with our findings, other investigators found that comorbidity was an important reason for non-enrollment of AD patients in clinical trials.(8;10)

Specific to our RCT with indomethacin was the exclusion of patients using particular drugs, such as aspirin, coumarin derivatives, ACE inhibitors, and loop diuretics. This might explain the reduced use of other cardiovascular drugs, such as digoxin and β-blockers, and lower occurrence of heart disease in the RCT group. Subsequently, the lower rate of comorbidity of the category "Endocrine/metabolic and breast" in the RCT group is caused by the lower incidence of diabetes mellitus, an important risk factor for cardiovascular comorbidity. This is also reflected by the lower concentration of serum glucose in the same group of patients.

Our study was limited by the number of patients in both the RCT group and the control group. However, the characteristics of AD patients in our RCT group are comparable with other RCTs, considering the mean age of participating patients and strict exclusion criteria of most RCTs. Furthermore, our observed differences in patient characteristics between groups are in conformity with two previous studies, that also investigated generalizability of AD trial results, yet using different study methods.(8;10)

In conclusion, the external validity or generalizability of the results of our RCT with indomethacin, and likely also of many other RCTs involving AD patients is limited at best. Due to strict eligibility criteria, subgroups of AD patients are formed with younger age, and less comorbidity and medication use, not representative of the AD patients seen in routine daily practice. Thus, clinicians should take this into account,

when prescribing AD medication. In addition, reports of RCTs should provide more detailed information concerning the characteristics of their participants. For future RCTs developing new AD therapies, we recommend less stringent eligibility criteria. This will also have the advantage of a higher enrollment rate into RCTs. Less stringent eligibility criteria will also lead to an increased adverse event rate during drug-trials, giving a better reflection of the side-effects one can expect prescribing a drug to the AD patient in daily clinical practice.

References

(1) Rothwell PM. External validity of randomised controlled trials: "to whom do the results of this trial apply?". Lancet 2005 Jan 1;365(9453):82-93.

(2) Juni P, Altman DG, Egger M. Systematic reviews in health care: Assessing the quality of controlled clinical trials. BMJ 2001 Jul 7;323(7303):42-46.

(3) Leber PD, Davis CS. Threats to the validity of clinical trials employing enrichment strategies for sample selection. Control Clin Trials 1998 Apr;19(2):178-187.

(4) Davis KL, Thal LJ, Gamzu ER, et al. A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disease. The Tacrine Collaborative Study Group. N Engl J Med 1992;327(18):1253-1259.

(5) van Dyck CH, Newhouse P, Falk WE, Mattes JA. Extended-release physostigmine in Alzheimer disease: a multicenter, double-blind, 12-week study with dose enrichment. Physostigmine Study Group. Arch Gen Psychiatry 2000 Feb;57(2):157-164.

(6) Zemlan FP. Velnacrine for the treatment of Alzheimer's disease: a double-blind, placebo-controlled trial. The Mentane Study Group. J Neural Transm 1996;103(8-9):1105-1116.

(7) Zemlan FP, Keys M, Richter RW, Strub RL. Double-blind placebo-controlled study of velnacrine in Alzheimer's disease. Life Sci 1996;58(21):1823-1832.

(8) Treves TA, Verchovsky R, Klimovitsky S, Korczyn AD. Recruitment rate to drug trials for dementia of the Alzheimer type. Alzheimer Dis Assoc Disord 2000 Oct;14(4):209-211.

(9) Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975 Nov;12(3):189-198.

(10) Schneider LS, Olin JT, Lyness SA, Chui HC. Eligibility of Alzheimer's disease clinic patients for clinical trials. J Am Geriatr Soc 1997 Aug;45(8):923-928.

(11) Schoenmaker N, van Gool WA. The age gap between patients in clinical studies and in the general population: a pitfall for dementia research. Lancet Neurol 2004 Oct;3(10):627-630.

(12) Warshaw G. Assessing physical status in Alzheimer disease research. Alzheimer Dis Assoc Disord 1997;11 Suppl 6:66-72.
(13) De Jong D, Jansen R, Hoefnagels W, et al. No effect of one-year treatment with indomethacin on Alzheimer's disease progression: a randomized controlled trial. PLoS ONE 2008;3(1):e1475.
(14) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984 Jul;34(7):939-944.
(15) Berg L. Clinical Dementia Rating (CDR). Psychopharmacol Bull 1988;24(4):637-639.
(16) Linn BS, Linn MW, Gurel L. Cumulative illness rating scale. J Am Geriatr Soc 1968 May;16(5):622-626.
(17) Miller MD, Paradis CF, Houck PR, et al. Rating chronic medical illness burden in geropsychiatric practice and research: application of the Cumulative Illness Rating Scale. Psychiatry Res 1992 Mar;41(3):237-248.
(18) Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993 Feb;43(2):250-260.
(19) Chappell AS, Gonzales C, Williams J, Witte MM, Mohs RC, Sperling R. AMPA potentiator treatment of cognitive deficits in Alzheimer disease. Neurology 2007 Mar 27;68(13):1008-1012.
(20) Johannsen P, Salmon E, Hampel H, et al. Assessing therapeutic efficacy in a progressive disease: a study of donepezil in Alzheimer's disease. CNS Drugs 2006;20(4):311-325.
(21) Kennedy J, Deberdt W, Siegal A, et al. Olanzapine does not enhance cognition in non-agitated and non-psychotic patients with mild to moderate Alzheimer's dementia. Int J Geriatr Psychiatry 2005 Nov;20(11):1020-1027.
(22) Sparks DL, Sabbagh MN, Connor DJ, et al. Atorvastatin for the treatment of mild to moderate Alzheimer disease: preliminary results. Arch Neurol 2005 May;62(5):753-757.
(23) Reines SA, Block GA, Morris JC, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 2004 Jan 13;62(1):66-71.
(24) Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 1999 Jul 13;53(1):197-201.
(25) Sun Y, Lu CJ, Chien KL, Chen ST, Chen RC. Efficacy of multivitamin supplementation containing vitamins B6 and B12 and folic acid as adjunctive treatment with a cholinesterase inhibitor in Alzheimer's disease: a 26-week, randomized, double-blind, placebo-con-

trolled study in Taiwanese patients. Clin Ther 2007 Oct;29(10):2204-2214.

(26) Imbimbo BP, Troetel WM, Martelli P, Lucchelli F. A 6-month, double-blind, placebo-controlled trial of eptastigmine in Alzheimer's disease. Dement Geriatr Cogn Disord 2000 Jan;11(1):17-24.

(27) Imbimbo BP, Martelli P, Troetel WM, Lucchelli F, Lucca U, Thal LJ. Efficacy and safety of eptastigmine for the treatment of patients with Alzheimer's disease [In Process Citation]. Neurology 1999 Mar 10;52(4):700-708.

(28) Gutzmann H, Hadler D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. J Neural Transm Suppl 1998;54:301-310.

(29) Antuono PG. Effectiveness and safety of velnacrine for the treatment of Alzheimer's disease. A double-blind, placebo-controlled study. Mentane Study Group. Arch Intern Med 1995 Sep 11;155(16):1766-1772.

(30) Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. Melissa officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. J Neurol Neurosurg Psychiatry 2003 Jul;74(7):863-866.

(31) Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. Salvia officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. J Clin Pharm Ther 2003 Feb;28(1):53-59.

(32) Rockwood K, Beattie BL, Eastwood MR, et al. A randomized, controlled trial of linopirdine in the treatment of Alzheimer's disease. Can J Neurol Sci 1997 May;24(2):140-145.

(33) Becker RE, Colliver JA, Markwell SJ, Moriearty PL, Unni LK, Vicari S. Double-blind, placebo-controlled study of metrifonate, an acetylcholinesterase inhibitor, for Alzheimer disease. Alzheimer Dis Assoc Disord 1996;10(3):124-131.

(34) Risner ME, Saunders AM, Altman JF, et al. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. Pharmacogenomics J 2006 Jul;6(4):246-254.

(35) Winblad B, Cummings J, Andreasen N, et al. A six-month double-blind, randomized, placebo-controlled study of a transdermal patch in Alzheimer's disease--rivastigmine patch versus capsule. Int J Geriatr Psychiatry 2007 May;22(5):456-467.

(36) Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol 2006 Oct;63(10):1402-1408.

(37) Wolkowitz OM, Kramer JH, Reus VI, et al. DHEA treatment of Alzheimer's disease: a random-

ized, double-blind, placebo-controlled study. Neurology 2003 Apr 8;60(7):1071-1076.

(38) Thal LJ, Ferguson JM, Mintzer J, Raskin A, Targum SD. A 24-week randomized trial of controlled-release physostigmine in patients with Alzheimer's disease. Neurology 1999 Apr 12;52(6):1146-1152.

(39) Feldman HH, Lane R. Rivastigmine: a placebo controlled trial of twice daily and three times daily regimens in patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry 2007 Oct;78(10):1056-1063.

(40) Rockwood K, Mintzer J, Truyen L, Wessel T, Wilkinson D. Effects of a flexible galantamine dose in Alzheimer's disease: a randomised, controlled trial. J Neurol Neurosurg Psychiatry 2001 Nov;71(5):589-595.

(41) Wilcock GK, Lilienfeld S, Gaens E. Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: multicentre randomised controlled trial. Galantamine International-1 Study Group. BMJ 2000 Dec 9;321(7274):1445-1449.

(42) Raskind MA, Peskind ER, Wessel T, Yuan W. Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension [In Process Citation]. Neurology 2000 Jun 27;54(12):2261-2268.

(43) Rosler M, Anand R, Cicin-Sain A, et al. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 1999 Mar 6;318(7184):633-638.

(44) Soininen H, West C, Robbins J, Niculescu L. Long-term efficacy and safety of celecoxib in Alzheimer's disease. Dement Geriatr Cogn Disord 2007;23(1):8-21.

(45) Alvarez XA, Cacabelos R, Laredo M, et al. A 24-week, double-blind, placebo-controlled study of three dosages of Cerebrolysin in patients with mild to moderate Alzheimer's disease. Eur J Neurol 2006 Jan;13(1):43-54.

(46) Doody RS, Gavrilova SI, Sano M, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. Lancet 2008 Jul 19;372(9634):207-215.

(47) Schneider LS, DeKosky ST, Farlow MR, Tariot PN, Hoerr R, Kieser M. A randomized, double-blind, placebo-controlled trial of two doses of Ginkgo biloba extract in dementia of the Alzheimer's type. Curr Alzheimer Res 2005 Dec;2(5):541-551.

(48) Brodaty H, Corey-Bloom J, Potocnik FC, Truyen L, Gold M, Damaraju CR. Galantamine prolonged-release formulation in the treatment of mild to moderate Alzheimer's disease. Dement Geriatr Cogn Disord 2005;20(2-3):120-132.

(49) Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group.

Neurology 2000 Jun 27;54(12):2269-2276.

(50) Peskind ER, Potkin SG, Pomara N, et al. Memantine treatment in mild to moderate Alzheimer disease: a 24-week randomized, controlled trial. Am J Geriatr Psychiatry 2006 Aug;14(8):704-715.

(51) Henderson VW, Paganini-Hill A, Miller BL, et al. Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. Neurology 2000 Jan 25;54(2):295-301.

(52) Sevigny JJ, Ryan JM, van Dyck CH, Peng Y, Lines CR, Nessly ML. Growth hormone secretagogue MK-677: no clinical effect on AD progression in a randomized trial. Neurology 2008 Nov 18;71(21):1702-1708.

(53) Aisen PS, Schneider LS, Sano M, et al. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. JAMA 2008 Oct 15;300(15):1774-1783.

Appendix

(1) Sevigny JJ, Ryan JM, van Dyck CH, Peng Y, Lines CR, Nessly ML. Growth hormone secretagogue MK-677: no clinical effect on AD progression in a randomized trial. Neurology 2008 Nov 18;71(21):1702-1708.

(2) Heo JH, Lee ST, Chu K, et al. An open-label trial of Korean red ginseng as an adjuvant treatment for cognitive impairment in patients with Alzheimer's disease. Eur J Neurol 2008 Aug;15(8):865-868.

(3) Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. Lancet Neurol 2008 Jun;7(6):483-493.

(4) Aisen PS, Schneider LS, Sano M, et al. High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. JAMA 2008 Oct 15;300(15):1774-1783.

(5) Doody RS, Gavrilova SI, Sano M, et al. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. Lancet 2008 Jul 19;372(9634):207-215.

(6) Bakchine S, Loft H. Memantine treatment in patients with mild to moderate Alzheimer's disease: results of a randomised, double-blind, placebo-controlled 6-month study. J Alzheimers Dis 2008 Feb;13(1):97-107.

(7) Porsteinsson AP, Grossberg GT, Mintzer J, Olin JT. Memantine treatment in patients with mild to moderate Alzheimer's disease already receiving a cholinesterase inhibitor: a randomized, double-blind, placebo-controlled trial. Curr Alzheimer Res 2008 Feb;5(1):83-89.

(8) Sun Y, Lu CJ, Chien KL, Chen ST, Chen RC. Efficacy of multivitamin supplementation containing vitamins B6 and B12 and folic acid as adjunctive treatment with a cholinesterase inhibitor in Alzheimer's disease: a 26-week, randomized, double-blind, placebo-controlled study in Taiwanese patients. Clin Ther 2007 Oct;29(10):2204-2214.

(9) Winblad B, Cummings J, Andreasen N, et al. A six-month double-blind, randomized, placebo-controlled study of a transdermal patch in Alzheimer's disease--rivastigmine patch versus capsule. Int J Geriatr Psychiatry 2007 May;22(5):456-467.

(10) Chappell AS, Gonzales C, Williams J, Witte MM, Mohs RC, Sperling R. AMPA potentiator treatment of cognitive deficits in Alzheimer disease. Neurology 2007 Mar 27;68(13):1008-1012.

(11) Feldman HH, Lane R. Rivastigmine: a placebo controlled trial of twice daily and three times daily regimens in patients with Alzheimer's disease. J Neurol Neurosurg Psychiatry

2007 Oct;78(10):1056-1063.

(12) Soininen H, West C, Robbins J, Niculescu L. Long-term efficacy and safety of celecoxib in Alzheimer's disease. Dement Geriatr Cogn Disord 2007;23(1):8-21.

(13) Freund-Levi Y, Eriksdotter-Jonhagen M, Cederholm T, et al. Omega-3 fatty acid treatment in 174 patients with mild to moderate Alzheimer disease: OmegAD study: a randomized double-blind trial. Arch Neurol 2006 Oct;63(10):1402-1408.

(14) Peskind ER, Potkin SG, Pomara N, et al. Memantine treatment in mild to moderate Alzheimer disease: a 24-week randomized, controlled trial. Am J Geriatr Psychiatry 2006 Aug;14(8):704-715.

(15) Johannsen P, Salmon E, Hampel H, et al. Assessing therapeutic efficacy in a progressive disease: a study of donepezil in Alzheimer's disease. CNS Drugs 2006;20(4):311-325.

(16) Risner ME, Saunders AM, Altman JF, et al. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. Pharmacogenomics J 2006 Jul;6(4):246-254.

(17) Alvarez XA, Cacabelos R, Laredo M, et al. A 24-week, double-blind, placebo-controlled study of three dosages of Cerebrolysin in patients with mild to moderate Alzheimer's disease. Eur J Neurol 2006 Jan;13(1):43-54.

(18) Schneider LS, DeKosky ST, Farlow MR, Tariot PN, Hoerr R, Kieser M. A randomized, double-blind, placebo-controlled trial of two doses of Ginkgo biloba extract in dementia of the Alzheimer's type. Curr Alzheimer Res 2005 Dec;2(5):541-551.

(19) Kennedy J, Deberdt W, Siegal A, et al. Olanzapine does not enhance cognition in non-agitated and non-psychotic patients with mild to moderate Alzheimer's dementia. Int J Geriatr Psychiatry 2005 Nov;20(11):1020-1027.

(20) Brodaty H, Corey-Bloom J, Potocnik FC, Truyen L, Gold M, Damaraju CR. Galantamine prolonged-release formulation in the treatment of mild to moderate Alzheimer's disease. Dement Geriatr Cogn Disord 2005;20(2-3):120-132.

(21) Sparks DL, Sabbagh MN, Connor DJ, et al. Atorvastatin for the treatment of mild to moderate Alzheimer disease: preliminary results. Arch Neurol 2005 May;62(5):753-757.

(22) Bilikiewicz A, Gaus W. Colostrinin (a naturally occurring, proline-rich, polypeptide mixture) in the treatment of Alzheimer's disease. J Alzheimers Dis 2004 Feb;6(1):17-26.

(23) Loeb MB, Molloy DW, Smieja M, et al. A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. J Am Geriatr Soc 2004 Mar;52(3):381-387.

(24) Reines SA, Block GA, Morris JC, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 2004 Jan 13;62(1):66-71.

(25) Thal LJ, Grundman M, Berg J, et al. Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. Neurology 2003 Dec 9;61(11):1498-1502.

(26) Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. Melissa officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomised, placebo controlled trial. J Neurol Neurosurg Psychiatry 2003 Jul;74(7):863-866.

(27) Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. JAMA 2003 Jun 4;289(21):2819-2826.

(28) Wolkowitz OM, Kramer JH, Reus VI, et al. DHEA treatment of Alzheimer's disease: a randomized, double-blind, placebo-controlled study. Neurology 2003 Apr 8;60(7):1071-1076.

(29) De Jesus Moreno MM. Cognitive improvement in mild to moderate Alzheimer's dementia after treatment with the acetylcholine precursor choline alfoscerate: a multicenter, double-blind, randomized, placebo-controlled trial. Clin Ther 2003 Jan;25(1):178-193.

(30) Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. Salvia officinalis extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. J Clin Pharm Ther 2003 Feb;28(1):53-59.

(31) Panisset M, Gauthier S, Moessler H, Windisch M. Cerebrolysin in Alzheimer's disease: a randomized, double-blind, placebo-controlled trial with a neurotrophic agent. J Neural Transm 2002 Jul;109(7-8):1089-1104.

(32) Aisen PS, Schmeidler J, Pasinetti GM. Randomized pilot study of nimesulide treatment in Alzheimer's disease. Neurology 2002 Apr 9;58(7):1050-1054.

(33) Dysken M, Kuskowski M, Love S. Ondansetron in the treatment of cognitive decline in Alzheimer dementia. Am J Geriatr Psychiatry 2002 Mar;10(2):212-215.

(34) Rockwood K, Mintzer J, Truyen L, Wessel T, Wilkinson D. Effects of a flexible galantamine dose in Alzheimer's disease: a randomised, controlled trial. J Neurol Neurosurg Psychiatry 2001 Nov;71(5):589-595.

(35) Wilkinson D, Murray J. Galantamine: a randomized, double-blind, dose comparison in patients with Alzheimer's disease. Int J Geriatr Psychiatry 2001 Sep;16(9):852-857.

(36) Ruether E, Husmann R, Kinzler E, et al. A 28-week, double-blind, placebo-controlled study with Cerebrolysin in patients with mild to moderate Alzheimer's disease. Int Clin Psychopharmacol 2001 Sep;16(5):253-263.

(37) Van Gool WA, Weinstein HC, Scheltens PK, Walstra GJ. Effect of hydroxychloroquine on progression of dementia in early Alzheimer's disease: an 18-month randomised, double-blind, placebo-controlled study. Lancet 2001 Aug 11;358(9280):455-460.

(38) Wilcock GK, Lilienfeld S, Gaens E. Efficacy and safety of galantamine in patients with mild to moderate Alzheimer's disease: multicentre randomised controlled trial. Galantamine

International-1 Study Group. BMJ 2000 Dec 9;321(7274):1445-1449.

(39) Raskind MA, Peskind ER, Wessel T, Yuan W. Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension [In Process Citation]. Neurology 2000 Jun 27;54(12):2261-2268.

(40) Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology 2000 Jun 27;54(12):2269-2276.

(41) Mulnard RA, Cotman CW, Kawas C, et al. Estrogen replacement therapy for treatment of mild to moderate Alzheimer disease: a randomized controlled trial. Alzheimer's Disease Cooperative Study. JAMA 2000 Feb 23;283(8):1007-1015.

(42) Aisen PS, Davis KL, Berg JD, et al. A randomized controlled trial of prednisone in Alzheimer's disease. Alzheimer's Disease Cooperative Study. Neurology 2000 Feb 8;54(3):588-593.

(43) Thal LJ, Forrest M, Loft H, Mengel H. Lu 25-109, a muscarinic agonist, fails to improve cognition in Alzheimer's disease. Lu25-109 Study Group. Neurology 2000 Jan 25;54(2):421-426.

(44) Henderson VW, Paganini-Hill A, Miller BL, et al. Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. Neurology 2000 Jan 25;54(2):295-301.

(45) Van Dyck CH, Newhouse P, Falk WE, Mattes JA. Extended-release physostigmine in Alzheimer disease: a multicenter, double-blind, 12-week study with dose enrichment. Physostigmine Study Group. Arch Gen Psychiatry 2000 Feb;57(2):157-164.

(46) Greenberg SM, Tennis MK, Brown LB, et al. Donepezil therapy in clinical practice: a randomized crossover study. Arch Neurol 2000 Jan;57(1):94-99.

(47) Imbimbo BP, Troetel WM, Martelli P, Lucchelli F. A 6-month, double-blind, placebo-controlled trial of eptastigmine in Alzheimer's disease. Dement Geriatr Cogn Disord 2000 Jan;11(1):17-24.

(48) Alvarez XA, Mouzo R, Pichel V, et al. Double-blind placebo-controlled study with citicoline in APOE genotyped Alzheimer's disease patients. Effects on cognitive performance, brain bioelectrical activity and cerebral perfusion. Methods Find Exp Clin Pharmacol 1999 Nov;21(9):633-644.

(49) Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 1999 Jul 13;53(1):197-201.

(50) Raskind MA, Cyrus PA, Ruzicka BB, Gulanski BI. The effects of metrifonate on the cognitive, behavioral, and functional performance of Alzheimer's disease patients. Metrifonate Study Group. J Clin Psychiatry 1999 May;60(5):318-325.

(51) Burns A, Rossor M, Hecker J, et al. The Effects of Donepezil in Alzheimer's Disease - Results from a Multinational Trial1. Dement Geriatr Cogn Disord 1999 May;10(3):237-244.

(52) Thal LJ, Ferguson JM, Mintzer J, Raskin A, Targum SD. A 24-week randomized trial of con-

trolled-release physostigmine in patients with Alzheimer's disease. Neurology 1999 Apr 12;52(6):1146-1152.

(53) Imbimbo BP, Martelli P, Troetel WM, Lucchelli F, Lucca U, Thal LJ. Efficacy and safety of eptastigmine for the treatment of patients with Alzheimer's disease [In Process Citation]. Neurology 1999 Mar 10;52(4):700-708.

(54) Rosler M, Anand R, Cicin-Sain A, et al. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 1999 Mar 6;318(7184):633-638.

(55) Imbimbo BP, Lucca U, Lucchelli F, Alberoni M, Thal LJ. A 25-week placebo-controlled study of eptastigmine in patients with Alzheimer disease. Alzheimer Dis Assoc Disord 1998 Dec;12(4):313-322.

(56) Gutzmann H, Hadler D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. J Neural Transm Suppl 1998;54:301-310.

(57) Morris JC, Cyrus PA, Orazem J, et al. Metrifonate benefits cognitive, behavioral, and global function in patients with Alzheimer's disease. Neurology 1998 May;50(5):1222-1230.

(58) Cummings JL, Cyrus PA, Bieber F, Mas J, Orazem J, Gulanski B. Metrifonate treatment of the cognitive deficits of Alzheimer's disease. Metrifonate Study Group. Neurology 1998 May;50(5):1214-1221.

(59) Rogers SL, Doody RS, Mohs RC, Friedhoff LT. Donepezil improves cognition and global function in Alzheimer disease: a 15-week, double-blind, placebo-controlled study. Donepezil Study Group. Arch Intern Med 1998 May 11;158(9):1021-1031.

(60) Becker RE, Colliver JA, Markwell SJ, Moriearty PL, Unni LK, Vicari S. Effects of metrifonate on cognitive decline in Alzheimer disease: a double-blind, placebo-controlled, 6-month study. Alzheimer Dis Assoc Disord 1998 Mar;12(1):54-57.

(61) Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. Neurology 1998 Jan;50(1):136-145.

(62) Rockwood K, Beattie BL, Eastwood MR, et al. A randomized, controlled trial of linopirdine in the treatment of Alzheimer's disease. Can J Neurol Sci 1997 May;24(2):140-145.

(63) Bodick NC, Offen WW, Levey AI, et al. Effects of xanomeline, a selective muscarinic receptor agonist, on cognitive function and behavioral symptoms in Alzheimer disease. Arch Neurol 1997 Apr;54(4):465-473.

(64) Weyer G, Babej-Dolle RM, Hadler D, Hofmann S, Herrmann WM. A controlled study of 2 doses of idebenone in the treatment of Alzheimer's disease. Neuropsychobiology 1997;36(2):73-82.

(65) Shrotriya RC, Cutler NR, Sramek JJ, Veroff AE, Hironaka DY. Efficacy and safety of BMY 21,502 in Alzheimer disease. Ann Pharmacother 1996 Dec;30(12):1376-1380.

(66) Rogers SL, Friedhoff LT. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US Multicentre, Randomized, Double-Blind, Placebo-Controlled Trial. The Donepezil Study Group. Dementia 1996 Nov;7(6):293-303.

(67) Thal LJ, Carta A, Clarke WR, et al. A 1-year multicenter placebo-controlled study of acetyl-L-carnitine in patients with Alzheimer's disease. Neurology 1996 Sep;47(3):705-711.

(68) Becker RE, Colliver JA, Markwell SJ, Moriearty PL, Unni LK, Vicari S. Double-blind, placebo-controlled study of metrifonate, an acetylcholinesterase inhibitor, for Alzheimer disease. Alzheimer Dis Assoc Disord 1996;10(3):124-131.

(69) Huff FJ, Antuono PG, Delagandara JE, et al. A treatment and withdrawal trial of besipirdine in Alzheimer disease. Alzheimer Dis Assoc Disord 1996;10(2):93-102.

(70) Antuono PG. Effectiveness and safety of velnacrine for the treatment of Alzheimer's disease. A double-blind, placebo-controlled study. Mentane Study Group. Arch Intern Med 1995 Sep 11;155(16):1766-1772.

(71) Knapp MJ, Knopman DS, Solomon PR, Pendlebury WW, Davis CS, Gracon SI. A 30-week randomized controlled trial of high-dose tacrine in patients with Alzheimer's disease. The Tacrine Study Group. JAMA 1994 Apr 6;271(13):985-991.

(72) Farlow M, Gracon SI, Hershey LA, Lewis KW, Sadowsky CH, Dolan-Ureno J. A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. JAMA 1992 Nov 11;268(18):2523-2529.

Chapter 3

Cerebrospinal fluid diagnosis in Alzheimer's disease

Chapter 3.1

The cerebrospinal fluid amyloid β_{42}/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia

D. de Jong
R.W.M.M. Jansen
H.P.H. Kremer
M.M. Verbeek

J Gerontol A Biol Sci Med Sci 2006;61(7):755-758

Abstract

Background. The differentiation of Alzheimer's disease (AD) from vascular dementia (VaD) is hampered by clinical diagnostic criteria with disappointing sensitivity and specificity. The objective of this study was to investigate whether cerebrospinal fluid (CSF) levels of total tau protein (t-tau), amyloid β_{42} protein ($A\beta_{42}$) and tau phosphorylated at threonine 181 (p-tau_{181}) are useful biomarkers to distinguish AD patients from VaD patients.

Methods. We measured CSF levels of p-tau_{181}, $A\beta_{42}$, and t-tau in 86 patients with a clinical diagnosis of AD and VaD, and 30 control subjects (NC).

Results. Optimal differentiation between AD and VaD was achieved by using the ratio of the CSF levels of $A\beta_{42}$ and p-tau_{181} (Q $A\beta_{42}$/p-tau) with sensitivity, specificity, positive and negative predictive values all ≥ 85%.

Conclusions. Our results support further efforts to prospectively validate the use of Q $A\beta_{42}$/p-tau as a biomarker to discriminate between AD and VaD.

Introduction

Differentiating of Alzheimer's disease (AD) from other dementia disorders, such as vascular dementia (VaD), is becoming increasingly important. An accurate and early diagnosis is essential for appropriate support and treatment of dementia patients, as symptomatic drugs are specifically available for AD patients and neuroprotective drugs based on altered amyloid β metabolism are being developed.

The clinical diagnostic criteria currently used for AD and VaD (1;2) have disappointing sensitivity and specificity,(3;4) often leading to the unequivocal diagnosis "mixed dementia", indicating clinical features of AD, but with multiple vascular lesions at brain imaging and/or cardiovascular risk factors. While AD and VaD are clearly different diseases (e.g. as exemplified by genetics), both seem to share vascular risk factors such as atherosclerosis and smoking.(5) Finally, AD may present with vascular comorbidity, which complicates the diagnostic work-up of AD patients. So, how to disentangle AD from vascular dementia?

Cerebrospinal fluid (CSF) analysis of amyloid β_{42} protein ($A\beta_{42}$) and total tau (t-tau), have been advocated as diagnostic biomarkers. T-tau levels are elevated and $A\beta_{42}$ levels decreased in CSF of AD patients compared to control subjects.(6;7) The combination of CSF t-tau and $A\beta_{42}$ yields a highly accurate differentiation between AD and normal controls (sensitivity 50 - 94%; specificity 83 - 100%).(6) However, CSF based differentiation of AD from VaD remains a challenge; specificity was only 48 % versus VaD.(8) Therefore, additional biomarkers are clearly needed. Quantification of hyperphosphorylated tau (p-tau) in CSF may be such a biomarker. CSF p-tau_{181} concentrations improve the discrimination of AD from dementia with Lewy bodies (DLB),(9) but its validity in discriminating AD from VaD has not extensively been studied.

In this retrospective case-control study we analyzed CSF levels of t-tau, $A\beta_{42}$ and p-tau_{181} of control subjects and patients with clinical AD and VaD, in order to achieve an optimal differentiation between AD and VaD.

Methods

Patients

Patients with mild to moderate AD (n = 61) and VaD (n = 25) were selected from a large database containing 260 patients with cognitive impairment or dementia of various origins (e.g. degenerative, vascular, hereditary, inflammatory, metabolic) who visited our outpatient clinic between 1992 and 2004. Only patients with a diagnosis of probable AD or VaD, according to accepted criteria,(1;2) were included. The standard diagnostic examination protocol included a complete geriatric assessment, neurological examination, neuropsychological testing, laboratory testing, imaging of the brain and a lumbar puncture. As controls (NC) we included thirty subjects over age 50 years who visited our outpatient clinic for various reasons but turned out not to suffer from a neurological disorder. Their CSF had normal leukocyte and erythrocyte counts, normal total protein, glucose and lactate concentrations, and no oligoclonal IgG bands.

CSF analysis

Lumbar punctures were performed after informed consent was obtained from the patients themselves and from the patient's legal representative. CSF from all participants was collected in polypropylene tubes, within 30 minutes transported to the adjacent laboratory at room temperature, centrifuged after routine investigations, and immediately aliquoted and stored at –80 C until analysis. Levels of t-tau, $A\beta_{42}$ and p-tau_{181} in CSF were measured using enzyme linked immunosorbent assays (all from Innogenetics NV, Gent, Belgium). In five AD and five VaD patients and ten control subjects, the amount of CSF was insufficient to measure the p-tau_{181} concentration.

Statistical analysis

Statistical procedures were performed using GraphPad Prism (San Diego, CA) software. All data were normally distributed; therefore, one-way ANOVA with Bonferroni's post hoc correction were used for multiple comparisons. Cut-off values, sensitivity and specificity for biomarkers in different groups were calculated using ROC curves. Cut-off value with the most optimal combination of sensitivity and specificity to

discriminate between these two groups for each biomarker were calculated. Subsequently, positive and negative predictive values (PPV and NPV) were calculated. Correlation analysis was performed by Pearson's method.

Results

Gender distribution was similar in the control (47% male, 53% female) and combined dementia patient groups (45% male, 55% female). The mean age of control subjects was significantly lower than of patients with AD and VaD ($p<.001$). There was no significant age difference between patients with AD and VaD (table 1).

Table 1

Age and levels of cerebrospinal fluid markers in patients and control subjects

	No. of patients (M/F)	Age (years)	$A\beta_{42}$ (pg/ml)	t-tau (pg/ml)	p-tau_{181} (pg/ml)	Q $A\beta_{42}$/ t-tau	Q $A\beta_{42}$/ p-tau
AD	25/36	68 ± 8.8*	419 ± 128†	613 ± 326†	103 ± 44†	0.9 ± 0.5†	4.9 ± 2.7†
VaD	14/11	72 ± 8.4*	655 ± 220‡	303 ± 307§	47 ± 14§	3.3 ± 1.9‡	15.9 ± 6.5§
NC	14/16	61 ± 8.3	869 ± 207	184 ± 89	53 ± 16	5.6 ± 2.4	16.5 ± 4.2

Notes: Mean ± SD. M = male, F = female. *$p<.001$, compared to NC. †$p<.001$, compared to VaD patients and NC. ‡$p<.001$, compared to AD patients and NC. §$p<.001$, compared to AD patients.

Mean CSF levels of $A\beta_{42}$ were significantly decreased in AD patients compared to VaD patients and control subjects ($p<.001$; tables 1 and 2) as well as in patients with VaD compared to control subjects ($p<.001$). Mean CSF levels of t-tau were significantly increased in AD patients compared to the other groups ($p<.001$). Only small, non-significant, differences were found between the mean CSF t-tau levels of patients with VaD and control subjects. Mean p-tau_{181} levels were significantly increased in CSF of patients with AD compared to patients with VaD ($p<.001$). There were no differences in mean CSF p-tau_{181} levels between patients with VaD and control subjects.

A positive correlation was observed between levels of p-tau$_{181}$ and t-tau in controls ($r = 0.88$, $p<.0001$) and in AD ($r = 0.84$, $p<.0001$), but not in VaD ($r = 0.31$, $p=0.2$).

Table 2

Discriminative value of cerebrospinal fluid markers between groups

Groups	Number of patients	Biomarkers and cutoff values	Sens (%)	Spec (%)	PPV (%)	NPV (%)
AD vs. VaD	61 vs. 25	Aβ$_{42}$ cutoff = 520 pg/ml *	82	76	89	63
	61 vs. 25	t-tau cutoff = 321 pg/ml *	80	76	89	61
	56 vs. 20	p-tau$_{181}$ cutoff = 68.5 pg/ml *	75	95	98	58
	61 vs. 25	Q Aβ$_{42}$/t-tau cutoff = 1.2 *	82	92	96	68
	56 vs. 20	Q Aβ$_{42}$/p-tau cutoff = 10.95 *	95	90	96	86
	56 vs. 20	Q Aβ$_{42}$/p-tau cutoff = 12.7 *	100	85	95	100
AD vs. NC	61 vs. 30	Aβ$_{42}$ cutoff = 603 pg/ml *	93	93	97	88
	61 vs. 30	t-tau cutoff = 352 pg/ml *	79	97	98	69
	56 vs. 20	p-tau$_{181}$ cutoff = 68 pg/ml *	75	85	93	55
	61 vs. 30	Q Aβ$_{42}$/t-tau cutoff = 1.895 *	95	97	98	91
	56 vs. 20	Q Aβ$_{42}$/p-tau cutoff = 8.7 *	89	95	98	76
	56 vs. 20	Q Aβ$_{42}$/p-tau cutoff = 13.2 *	100	85	95	100
VaD vs. NC	25 vs. 30	Aβ$_{42}$ cutoff = 814 pg/ml *	80	67	67	80
	25 vs. 30	t-tau cutoff = 174.5 pg/ml	76	57	59	74
	20 vs. 20	p-tau$_{181}$ cutoff = 45.5 pg/ml	55	70	65	61
	25 vs. 30	Q Aβ$_{42}$/t-tau cutoff = 3.5 *	68	87	81	76
	20 vs. 20	Q Aβ$_{42}$/p-tau cutoff = 14.15	55	80	73	64

Notes: Sensitivity (Sens) and specificity (Spec) to differentiate between Alzheimer's disease (AD), vascular dementia (VaD) and control subjects (NC) are listed, using different cerebrospinal fluid markers and cutoff values. In addition, positive predictive values (PPV) and negative predictive values (NPV) are shown. * $p<.001$.

The $A\beta_{42}$/p-tau_{181} ratio (Q $A\beta_{42}$/p-tau) was significantly lower ($p<.001$) in patients with AD compared to control subjects and patients with VaD. Furthermore, the $A\beta_{42}$/t-tau ratio (Q $A\beta_{42}$/t-tau) was significantly different in all three studied groups (tables 1 and 2).

High sensitivity and specificity for the discrimination between AD and VaD was obtained at two optimal cutoff levels of Q $A\beta_{42}$/p-tau (table 2). At a cutoff level of 12.7, sensitivity was 100% and specificity 85% with a PPV of 95% and a NPV of 100%. At a slightly lower cutoff level (10.95), sensitivity and specificity were 95% and 90%, respectively (PPV 96% and NPV 86%). Sensitivity and specificity of any of the other biomarkers was inferior compared to Q$A\beta_{42}$ / p-tau_{181}.

Optimal separation of the AD and control group using Q$A\beta_{42}$/t-tau was achieved at a cut-off level of 1.895, with a sensitivity of 95% and specificity of 97%. In addition, very high PPV (95%) and NPV (100%) was reached with Q$A\beta_{42}$/p-tau at a cutoff level of 13.2. Discrimination between VaD and controls using a Q$A\beta_{42}$/t-tau cut-off value of 3.5 resulted in a combination of 68% sensitivity and 87% specificity only.

Discussion

In the present study, we found decreased CSF $A\beta_{42}$ levels and increased CSF t-tau levels in patients with AD compared to VaD and normal subjects, consistent with the literature.(6-10) Also, the levels of $A\beta_{42}$ and tau in patients with VaD are in line with another study.(8) Our main finding, however, is that the ratio of $A\beta_{42}$ to p-tau_{181} (Q $A\beta_{42}$/p-tau) distinguishes between AD and VaD patients with high discriminatory power. Mean p-tau_{181} levels are doubled in the AD group, but normal in VaD, similar to other observations.(11;12) Identical results were found using tau protein phosphorylated at serine 199 (p-tau_{199}) and threonine 231 (p-tau_{231}).(9;11;13)

Q $A\beta_{42}$/p-tau was significantly decreased in the AD group compared to the VaD group. Previously it has been reported that in AD patients a low $A\beta_{42}$/p-tau_{181} ratio was observed compared to healthy controls, patients with non-AD dementias and patients with other neurological disorders.(14) In this study we demonstrated that Q $A\beta_{42}$/p-tau has excellent diagnostic value in the differentiation of AD from VaD. According to a consensus report (15) a useful biomarker should be reliable,

reproducible, and have both a sensitivity and a PPV greater than 80% for detecting AD and a specificity greater than 80% for distinguishing other dementias. Q $A\beta_{42}$/p-tau fulfills these requirements, since sensitivity, specificity, PPV and NPV are all well above 85%. For the discrimination between AD and NC groups, Q $A\beta_{42}$/t-tau may also be a useful biomarker, since at a cutoff level of 1.895 high sensitivity, specificity, PPV and NPV (each 91% or higher) were obtained. Since all patients' diagnoses were based on clinical criteria and not neuropathologically confirmed, improvement of sensitivity and specificity awaits prospective neuropathology supported studies.

A potential confounder of this study is the age-difference between patients and controls. However, t-tau is the only CSF biomarker known to be positively correlated with age ($r = 0.60$, $p<.001$).(16) Thus, differences in mean CSF t-tau levels between NC and AD might be smaller than described before. Since the results of our study do not suggest an important role for the analysis of t-tau in the discrimination of AD from VaD, and because there was no significant age-difference between AD and VaD, this finding does not affect our main results.

In the VaD patients group, one patient had exceptional high tau levels (1603 pg/ml) with normal p-tau_{181} levels (56 pg/ml). This patient underwent the lumbar puncture only five weeks after he suffered from a stroke - a known cause of a transient (3-5 months) increase in CSF t-tau, but not of p-tau.(17)

In many previous studies the focus of the application of CSF biomarkers was the differentiation between AD and NC. Only few addressed the truly relevant discrimination between AD and other dementia disorders, particularly VaD. As recent studies suggested that vascular risk factors, including atherosclerosis, diabetes and smoking, might significantly contribute to the pathogenesis of AD,(5;18) the conventional distinction between AD and VaD has become controversial. Our study suggests that a) there are biological differences between what we call AD and VaD; and b) Q $A\beta_{42}$/p-tau in CSF may detect such differences in relevant clinical situations. The true contribution of $A\beta_{42}$/p-tau CSF assessments to the clinical management of patients with late onset dementia disorders remains to be established, preferably through a prospective randomized masked validation study with appropriate control populations.

References

(1) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984 Jul;34(7):939-944.

(2) Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993 Feb;43(2):250-260.

(3) Lopez OL, Litvan I, Catt KE, et al. Accuracy of four clinical diagnostic criteria for the diagnosis of neurodegenerative dementias. Neurology 1999 Oct 12;53(6):1292-1299.

(4) Gold G, Bouras C, Canuto A, et al. Clinicopathological validation study of four sets of clinical criteria for vascular dementia. Am J Psychiatry 2002 Jan;159(1):82-87.

(5) Breteler MM. Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. Neurobiol Aging 2000 Mar;21(2):153-160.

(6) Verbeek MM, De Jong D, Kremer HP. Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases. Ann Clin Biochem 2003 Jan;40(Pt 1):25-40.

(7) Clark CM, Xie S, Chittams J, et al. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? Arch Neurol 2003 Dec;60(12):1696-1702.

(8) Andreasen N, Minthon L, Davidsson P, et al. Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol 2001 Mar;58(3):373-379.

(9) Itoh N, Arai H, Urakami K, et al. Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease. Ann Neurol 2001 Aug;50(2):150-156.

(10) Leszek J, Malyszczak K, Janicka B, Kiejna A, Wiak A. Total tau in cerebrospinal fluid differentiates Alzheimer's disease from vascular dementia. Med Sci Monit 2003 Nov;9(11):CR-84-CR488.

(11) Hampel H, Buerger K, Zinkowski R, et al. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch Gen Psychiatry 2004 Jan;61(1):95-102.

(12) Schonknecht P, Pantel J, Hunt A, et al. Levels of total tau and tau protein phosphorylated at threonine 181 in patients with incipient and manifest Alzheimer's disease. Neurosci Lett 2003 Mar 20;339(2):172-174.

(13) Buerger K, Zinkowski R, Teipel SJ, et al. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. Arch Neurol 2002 Aug;59(8):1267-1272.

(14) Maddalena A, Papassotiropoulos A, Muller-Tillmanns B, et al. Biochemical diagnosis of Alzheimer disease by measuring the cerebrospinal fluid ratio of phosphorylated tau protein to beta-amyloid peptide42. Arch Neurol 2003 Sep;60(9):1202-1206.

(15) Consensus report of the Working Group on: "Molecular and Biochemical Markers of Alzheimer's Disease". The Ronald and Nancy Reagan Research Institute of the Alzheimer's Association and the National Institute on Aging Working Group. Neurobiol Aging 1998 Mar;19(2):109-116.

(16) Sjogren M, Vanderstichele H, Agren H, et al. Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values. Clin Chem 2001 Oct;47(10):1776-1781.

(17) Hesse C, Rosengren L, Andreasen N, et al. Transient increase in total tau but not phospho-tau in human cerebrospinal fluid after acute stroke. Neurosci Lett 2001 Jan 19;297(3):187-190.

(18) de la Torre JC. Is Alzheimer's disease a neurodegenerative or a vascular disorder? Data, dogma, and dialectics. Lancet Neurol 2004 Mar;3(3):184-190.

Chapter 3.2

CSF neurofilament proteins in the differential diagnosis of dementia

D. de Jong
R.W.M.M. Jansen
Y.A.L. Pijnenburg
W.J.A. van Geel
G.F. Borm
H.P.H. Kremer
M.M. Verbeek

J Neurol Neurosurg Psychiatry 2007 Sep;78(9):936-8

Abstract

Background: Neurofilament (NF) proteins are major cytoskeletal constituents of neurons. Increased CSF NF levels may reflect neuronal degeneration.

Objective: To investigate the diagnostic value of CSF NF analysis to discriminate in relatively young dementia patients between frontotemporal lobe degeneration (FTLD) and early-onset Alzheimer disease (EAD; onset ≤ 65 years of age), and in elderly dementia patients between dementia with Lewy bodies (DLB) and late-onset AD (LAD; onset > 65 years of age).

Methods: In CSF of 28 FTLD, 37 EAD, 18 DLB, and 33 LAD patients, and 26 control subjects, we analysed NF light chain (NFL), phosphorylated NF heavy chain (pNFH), amyloid β_{42} protein ($A\beta_{42}$), total tau (t-tau), and tau phosphorylated at threonine 181 (p-tau_{181}).

Results: CSF NFL levels were higher in FTLD patients compared to EAD patients ($p < 0.001$), and diagnostic accuracy of p-tau_{181} and $A\beta_{42}$ analysis improved with addition of NFL analysis (sensitivity 86%, specificity 100%). CSF pNFH levels were elevated in DLB, LAD, and FTLD compared to controls ($p < 0.05$), however, no significant differences were found between the dementia groups.

Conclusions: In the diagnostic workup of relatively young dementia patients, CSF NFL levels may play a role in the discrimination between FTLD and EAD, especially in combination with $A\beta_{42}$ and p-tau_{181} analysis.

Introduction

The clinical differentiation between Alzheimer disease (AD), frontotemporal lobar degeneration (FTLD) and dementia with Lewy bodies (DLB) can be achieved using a combination of clinical criteria, neuroimaging, and CSF biomarkers, in particular amyloid β_{42} protein ($A\beta_{42}$), total tau (t-tau) and phosphorylated tau (p-tau).(1) However, in younger patients with incipient or mild dementia, it is often still difficult to discriminate between AD and FTLD. Especially so, as in patients with early-onset AD (EAD) focal cortical symptoms (language, praxis or executive function problems) and behavioural deficits, can be more prominent than memory dysfunction.(2) A similar challenge exists in older patients for the differentiation between AD and DLB, since neuropsychiatric symptoms and extrapyramidal signs are commonly seen in AD patients with more advanced disease.(3)

Neurofilament (NF) proteins are major constituents of the neuronal cytoskeleton. Localized in large neurons and myelinated axons, they play an important role in neuronal structure. NFs consist of three polypeptides; the light (NFL), medium (NFM) and heavy (NFH) subunits.(4) Increased levels of NFs in CSF may reflect neuronal degeneration in neurological disease.

The analyses of NFs in CSF of dementia patients challenged various researchers. CSF NFL levels were increased in AD, late-onset AD (LAD), and FTLD compared to controls, and tended to be increased in FTLD compared to EAD.(5-10) A positive correlation between CSF NFL levels and the degree of cognitive impairment was found in FTLD and LAD.(9)

Less is known about CSF levels of the other NF subunits. One study described increased CSF levels of phosphorylated NFH/M in AD compared to vascular dementia (VaD) and controls.(5) Others found elevated CSF NFH levels in AD and VaD compared to controls, but no differences between FTLD and controls, or between AD, VaD, and FTLD.(11)

We assumed that widespread neuronal degeneration leads to elevated NF levels in CSF, and therefore studied CSF levels of NFL and phosphorylated NFH (pNFH) in patients with neurodegenerative dementias. We investigated whether CSF NF protein analysis helps to discriminate between FTLD and EAD in relatively

young dementia patients, and between DLB and LAD in older dementia patients, and whether it has superior or additional diagnostic value compared to $A\beta_{42}$, t-tau, and p-tau analysis.

Methods

Patients

This retrospective study included 37 EAD (onset before or at 65 years of age), 33 LAD (onset after 65 years of age), 18 DLB, and 28 FTLD patients, identified through the CSF databases of the Radboud University Nijmegen Medical Centre, and the VU Medical Centre, Amsterdam. Only patients with a probable diagnosis according to the accepted clinical diagnostic criteria, were included.(12-14) The standard diagnostic examination protocol included medical history, physical and neurological examination, neuropsychological testing, laboratory testing, brain imaging, and a lumbar puncture.

Twenty-six control subjects were included who underwent a lumbar puncture for various reasons, but did not have a neurological disorder.

CSF analysis

Lumbar punctures were performed after informed consent was obtained from the patient or the legal representative. CSF was collected in polypropylene tubes, within 30 minutes transported to the adjacent laboratory, centrifuged after routine investigations, and immediately aliquoted and stored at –80°C until analysis.

Determination of NFL levels was performed using our previously described sandwich ELISA.(15) Levels of pNFH were determined using a modified version of a sandwich ELISA developed by others,(16) that we recently described in more detail. (17) T-tau, $A\beta_{42}$ and p-tau_{181} were measured using ELISA (Innogenetics NV, Gent, Belgium).

In two controls, three EAD, four LAD, and one DLB patient, the CSF amount was insufficient to measure either pNFH or p-tau_{181} concentration.

Statistical analysis

CSF NFL and pNFH levels followed a lognormal distribution, so the statistical analysis was carried out on log transformed values. Tukey's method for multiple comparisons was used for group comparisons. In additional analyses, age and gender were included as covariates. For each CSF marker, the area under the receiver operating characteristic curve (AUC), cut-off values, sensitivity, and specificity were calculated. Logistic regression with backwards selection was used to derive combinations of CSF markers with the highest diagnostic value. For correlations Spearman's rank coefficient was used.

Results

Patient characteristics are listed in table 1. Our main study groups were matched for age. Median age of controls was significantly lower compared to LAD and DLB patients. Both age and gender were included in the statistical analysis, but did not substantially change the results.

CSF NFL levels were significantly higher in FTLD compared to EAD and controls, but were comparable in DLB and LAD (table 1). CSF pNFH levels were significantly elevated in LAD, FTLD, and DLB compared to controls (table 1). Furthermore, CSF pNFH levels were significantly higher in DLB than in EAD, but no differences were found between DLB and LAD, or between FTLD and EAD. In none of the dementia groups or dementia patients as a whole, a significant correlation of CSF NFL or pNFH with MMSE score, disease duration, or age was found.

In FTLD compared to EAD, CSF $A\beta_{42}$ was significantly higher, and t-tau and p-tau_{181} lower (table 1). CSF $A\beta_{42}$ levels were comparable in DLB and LAD. CSF levels of t-tau and p-tau_{181} in DLB were significantly lower compared to LAD.

Table 1

Clinical characteristics and levels of CSF markers

Parameter	EAD	FTLD	LAD	DLB	Controls
Age, years	61 (52–69)	63 (43–79)	76 (69–90)	72 (58–90)	60 (53–85)
No. of patients, male/female	37 (15/22)	28 (20/8)	33 (13/20)	18 (13/5)	26 (12/14)
Disease duration, years	3.0 (1.0–10.0)	3.0 (1.0–10.0)	2.0 (0.5–7.0)	1,5 (1.0–5.0)	
MMSE score	20 (6–28)	24 (3–28)	21 (9–27)	23 (2–28)	
NFL, pg/ml	6.1 (n=37)[1] (0.0–40.3)	16.9 (n=28)[2,3] (0.0–76.4)	15.2 (n=33) (0.0–70.1)	10.4 (n=18) (0.0–60.4)	5.0 (n=26) (0.0–33.8)
pNFH, pg/ml	88 (n=36)[4] (39-205)	109 (n=28)[5] (52–373)	124 (n=29)[5] (49–398)	131 (n=18)[2,3] (71–711)	84 (n=24) (38–112)
$A\beta_{42}$, pg/ml	365 (n=37)[6] (184–703)	582 (n=28)[4,7,8] (202–1408)	419 (n=33)[6] (197–873)	444 (n=18)[1] (176–784)	-
t-tau, pg/ml	565 (n=37)[1,4] (173–1946)	362 (n=28)[3,10] (115–983)	647 (n=33)[1,9] (178–2400)	270 (n=18)[3,8] (105–961)	-
p-tau_{181}, pg/ml	86 (n=35)[6,9] (47–250)	51 (n=28)[7,8] (24–132)	89 (n=33)[6,9] (31–254)	58 (n=17)[7,8] (32–89)	-

Values are expressed as medians (range). [1]p<0.01, compared to FTLD; [2]p<0.001, compared to controls; [3]p<0.01, compared to EAD; [4]p<0.01, compared to DLB; [5]p<0.05, compared to controls; [6]p<0.001, compared to FTLD; [7]p<0.001, compared to EAD; [8]p<0.001, compared to LAD; [9]p<0.001, compared to DLB; [10]p<0.01, compared to LAD.

When discriminating between FTLD and EAD using CSF NFL levels, sensitivity was 82%, and specificity 70% (table 2; AUC = 0.80). This discriminative value was comparable with $A\beta_{42}$ and p-tau_{181}. However, a combination of CSF levels of $A\beta_{42}$ and p-tau_{181} improved sensitivity and specificity significantly compared to p-tau_{181} alone (AUC = 0.89), and even more so when CSF NFL levels were added (AUC = 0.92). CSF NFH levels did not offer additional diagnostic value.

Table 2

Discriminative value of CSF markers between patient groups

	FTLD versus EAD				DLB versus LAD			
CSF markers	AUC	Cut off (pg/ml)	Sens (%)	Spec (%)	AUC	Cut off (pg/ml)	Sens (%)	Spec (%)
NFL	0.80	6.7	82	70	0.53	6.0	33	82
pNFH	0.62	129	46	78	0.55	84.8	89	28
$A\beta_{42}$	0.78	488	64	92	0.49	590	89	21
t-tau	0.71	420	68	70	0.82	361	72	88
p-tau_{181}	0.81	53.0	57	97	0.86	68.7	82	85
$A\beta_{42}$ + p-tau_{181}	0.89*	-8.1[1]	75	94	0.88	22.5[3]	82	94
$A\beta_{42}$ + p-tau_{181} + NFL	0.92**	-4.1[2]	86	100	0.88	23.0[4]	82	94

AUC, area under the curve; Sens, sensitivity; Spec, specificity.
*statistically significant improvement versus p-tau_{181} alone;
**statistically significant improvement versus p-tau_{181} and $A\beta_{42}$ combination
The discriminant formulas for the combined markers are: [1] 2.6*ln(ptau_{181})-3.0*ln($A\beta_{42}$);
[2] 3.5*ln(ptau_{181})-2.3*ln($A\beta_{42}$)-2.0*ln(NFL); [3] 3.8*ln(ptau_{181})+1.0*ln($A\beta_{42}$);
[4] 3.9*ln(ptau_{181})+1.0*ln($A\beta_{42}$)+0.1*ln(NFL).

When differentiating DLB from LAD, the combination of CSF p-tau_{181} and $A\beta_{42}$ performed best (table 2). CSF NFL or pNFH measurements did not have additional discriminative value.

Discussion

In FTLD, CSF NFL levels were increased, consistent with other studies,(6;9;10) and the analysis had additional diagnostic value in the differentiation of FTLD from EAD, in combination with p-tau_{181} and $A\beta_{42}$. In contrast to a recent study, we also found elevated CSF pNFH levels in FTLD.(11) Thus, perhaps not only tau, but also other cytoskeleton proteins are involved in the pathophysiology of FTLD.(9;10) The heterogeneity in the underlying pathology of FTLD, however, makes it difficult to

disentangle the exact function of NFs in this process.(18) It has been hypothesised that NFs are either defective or overexpressed in FTLD and aggregate intracellularly, disrupting the cytoskeleton and cell integrity, causing cytoskeleton protein leakage into the CSF, and premature cell death.(9) Since only CSF levels of NFL and pNFH, but not tau, are increased, this suggests that NFs are overexpressed in neurons, and that these neurons are selectively vulnerable in FTLD.

In DLB compared to LAD, CSF levels of NFL and pNFH had no discriminative value. Nevertheless, our observation of elevated pNFH levels in DLB contributes to the emerging picture of abnormal CSF protein composition in degenerative dementias. Since CSF NFL levels were not increased in DLB, the difference in CSF NF and pNFH composition compared to FTLD suggests differences in the underlying pathophysiologic mechanism of these disorders. There is evidence that phosphorylated and nonphosphorylated NFs accumulate in Lewy bodies. Also, an increased number of cortical NF-containing neurons was observed in DLB, compared to AD and controls,(19) which apparently does not lead to increased CSF NFL concentrations. Altogether this suggests that in DLB, AD, and FTLD, different cortical neuronal populations are affected, and different subsets of NFs are involved.

In LAD, we observed elevated CSF pNFH levels, corroborating earlier observations.(11) We could not confirm previous reports that showed increased CSF NFL levels in AD.(5-9;20) Unanticipated, a nonsignificant trend of increased CSF NFL and pNFH levels in LAD compared to EAD was found, consistent with another study describing elevated CSF NFL levels in LAD compared to EAD.(9) Since an association between the presence of white matter changes and increased CSF NFL was previously described,(8) and white matter lesions are more common in LAD than EAD patients, this might explain our observation.

The implications of our study may be limited because of the relatively small numbers of patients. This, together with the absence of postmortem verification, might explain the considerable dispersion in CSF NFL and pNFH levels found in each patient group. Nevertheless, compared to previous studies on NFs in CSF, our FTLD group is the largest, and our DLB group is the first ever described. Also, the absence of postmortem verification applies to many studies on biomarkers in dementia. To compensate for the lack of postmortem diagnoses, we conducted an extensive diagnostic examination protocol.

We conclude that measurement of CSF NFL levels can play a role in the diagnostic workup of patients with FTLD and EAD, particularly in combination with $A\beta_{42}$ and p-tau_{181} analysis. The contribution of CSF NFL analysis to the clinical management of these relatively young dementia patients will have to be established through larger prospective randomized masked validation studies. In addition, ongoing research may shed more light on pathophysiological mechanisms explaining the observed elevated CSF levels of NFL in FTLD, and pNFH in FTLD, LAD, and DLB.

References

(1) De Jong D, Jansen RWMM, Kremer HPH, Verbeek MM. Cerebrospinal fluid amyloid β42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia. J Gerontol A Biol Sci Med Sci 2006;61(7):755-758.

(2) Greicius MD, Geschwind MD, Miller BL. Presenile dementia syndromes: an update on taxonomy and diagnosis. J Neurol Neurosurg Psychiatry 2002 Jun;72(6):691-700.

(3) Burns JM, Galvin JE, Roe CM, Morris JC, McKeel DW. The pathology of the substantia nigra in Alzheimer disease with extrapyramidal signs. Neurology 2005 Apr 26;64(8):1397-1403.

(4) Petzold A. Neurofilament phosphoforms: surrogate markers for axonal injury, degeneration and loss. J Neurol Sci 2005 Jun 15;233(1-2):183-198.

(5) Hu YY, He SS, Wang XC, et al. Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer disease patients. Neurosci Lett 2002 Mar 8;320(3):156-160.

(6) Rosengren LE, Karlsson JE, Sjogren M, Blennow K, Wallin A. Neurofilament protein levels in CSF are increased in dementia. Neurology 1999 Mar 23;52(5):1090-1093.

(7) Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem 1996 Nov;67(5):2013-2018.

(8) Sjogren M, Blomberg M, Jonsson M, et al. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. J Neurosci Res 2001 Nov 1;66(3):510-516.

(9) Sjogren M, Rosengren L, Minthon L, Davidsson P, Blennow K, Wallin A. Cytoskeleton proteins in CSF distinguish frontotemporal dementia from AD. Neurology 2000 May 23;54(10):1960-1964.

(10) Sjogren M, Wallin A. Pathophysiological aspects of frontotemporal dementia--emphasis on cytoskeleton proteins and autoimmunity. Mech Ageing Dev 2001 Nov;122(16):1923-1935.

(11) Brettschneider J, Petzold A, Schottle D, Claus A, Riepe M, Tumani H. The neurofilament heavy chain (NfH) in the cerebrospinal fluid diagnosis of Alzheimer's disease. Dement Geriatr Cogn Disord 2006;21(5-6):291-295.

(12) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984 Jul;34(7):939-944.

(13) McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996 Nov;47(5):1113-1124.

(14) Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998 Dec;51(6):1546-1554.

(15) Van Geel WJ, Rosengren LE, Verbeek MM. An enzyme immunoassay to quantify neurofilament light chain in cerebrospinal fluid. J Immunol Methods 2005 Jan;296(1-2):179-185.

(16) Petzold A, Keir G, Green AJ, Giovannoni G, Thompson EJ. A specific ELISA for measuring neurofilament heavy chain phosphoforms. J Immunol Methods 2003 Jul;278(1-2):179-190.

(17) Abdo WF, van de Warrenburg BP, Munneke M, et al. CSF analysis differentiates multiple-system atrophy from idiopathic late-onset cerebellar ataxia. Neurology 2006 Aug 8;67(3):474-479.

(18) Bergmann M, Kuchelmeister K, Schmid KW, Kretzschmar HA, Schroder R. Different variants of frontotemporal dementia: a neuropathological and immunohistochemical study. Acta Neuropathol (Berl) 1996 Aug;92(2):170-179.

(19) Shepherd CE, McCann H, Thiel E, Halliday GM. Neurofilament-immunoreactive neurons in Alzheimer's disease and dementia with Lewy bodies. Neurobiol Dis 2002 Mar;9(2):249-257.

(20) Norgren N, Rosengren L, Stigbrand T. Elevated neurofilament levels in neurological diseases. Brain Res 2003 Oct 10;987(1):25-31.

Chapter 3.3

Current state and future directions of neurochemical biomarkers for Alzheimer's disease (review)

D. de Jong
H.P.H. Kremer
M.G.M. Olde Rikkert
M.M. Verbeek

Clin Chem Lab Med 2007;45(11):1421-1434

Abstract

In this comprehensive review we summarize the current state-of-the-art of neurochemical biomarkers for Alzheimer's disease. Predominantly these biomarkers comprise cerebrospinal fluid biomarkers directly related to the pathofysiology of this disorder (such as amyloid β protein, tau protein). We particularly pay attention to the innovations in this area that have been made in technological aspects during the past 5 years (e.g. multiplex analysis of biomarkers, proteomics), to the discovery of novel, potential biomarkers (e.g amyloid β oligomers, isoprostanes), and to the extension of this research towards identification of biomarkers in plasma.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles (NFTs) in cortical and limbic brain regions (1;2). Until now, a definitive diagnosis of Alzheimer's disease can only be made after postmortem examination of the patient. During life only a "probable AD" diagnosis is possible, as based on clinical features and the results of neurological and neuropsychological testing, added by the exclusion of other dementias, in particular frontotemporal lobe degeneration (FTLD), dementia with Lewy bodies (DLB), and vascular dementia (VaD). An accurate and early diagnosis is essential for appropriate support and treatment of dementia patients, since drugs for the symptomatic treatment of AD are currently available and drugs that may slow or halt the progression of the disease are being developed.

The clinical diagnostic criteria currently used for dementia disorders (NINCDS-ADRDA criteria for AD, Lund and Manchester criteria for FTLD, Consensus guidelines for DLB and NINDS-AIREN criteria for VaD (3-6)) were initially developed for research purposes. The accuracy of these criteria was then examined in several clinicopathological studies. It was established that a clinical diagnosis of probable AD could be achieved with a sensitivity of 41% to 93% and a specificity varying between 23% and 100% (7-9). Similar studies yielded 85% sensitivity and 97% specificity for FTLD (9), 57 to 78% sensitivity and 64 to 100% specificity for probable DLB (9-11), and 20% sensitivity and 93% specificity for probable VaD (12). Clearly, to improve upon these less than perfect characteristics, the introduction of additional discriminating diagnostic markers (biomarkers) is needed to improve our diagnostic accuracy. Analysis of various brain specific proteins in cerebrospinal fluid (CSF), i.e. biomarkers closest to the brain's chemical composition, have already proven to be very sensitive and specific in preliminary validation studies (13-16).

In this review, we summarize the studies reporting on CSF biomarkers in AD and other dementias. Furthermore, we particularly pay attention to the technological innovations in this area during the past five years, to the discovery of novel, potential biomarkers, and to the extension of this research towards identification of biomarkers in plasma.

Cerebrospinal fluid $A\beta_{42}$, t-tau and p-tau

In this paragraph we summarize the abundant literature that has been published over the past ten years on this topic. For a more extensive review of this topic we refer to previously published reviews (17-20).

$A\beta_{42}$, t-tau and p-tau in the nosological classification of dementia disorders

The characteristic neuropathological findings in AD brains are senile plaques, NFTs, and degeneration of neurons and their synapses. The major component of senile plaques is amyloid β protein (Aβ), while tau protein, particularly hyperphosphorylated tau, is the primary constituent of NFTs. Therefore, levels of both proteins in CSF of dementia patients have been thoroughly investigated as potential diagnostic biomarkers.

Significantly decreased concentrations of $A\beta_{42}$ in CSF were found in AD patients compared to controls (15;21-27), to patients with VaD (25), and to patients with FTLD (27) (Table 1). Mean CSF $A\beta_{42}$ levels were decreased in patients with DLB compared to normal subjects, but no differences were found between the AD and the DLB group (26). It should be noted, however, that in none of these studies a clear-cut discrimination between the various forms of dementia could be achieved.

The level of CSF total tau protein (t-tau), which includes both normal and hyperphosphorylated tau (p-tau), seems to correlate with the number of NFTs in AD postmortem brains (28). Mean CSF t-tau levels were found significantly elevated in AD patients as compared to control subjects (15;23-27;29-35), DLB patients (25;26;32), FTLD patients (27;30), and patients with VaD (25;30;33) (Table 1). In FTLD patients, however, a large variation in CSF t-tau levels is described in different studies, varying from non-detectable to significantly elevated (27;30;34;36-41). This is most likely due to the fact that FTLD comprises at least three neuropathologically different subtypes, that is with tau deposition (Pick's disease and familiar FTLD with tau pathology, both approximately 20% of FTLD patients) or without tau pathology (60% of FTLD patients) (42;43). Some of these tau-negative FTLD patients are recently linked to mutations in the progranulin gene (44;45). Furthermore, the surprisingly low CSF tau levels found in one study (40) can also be explained by methodological issues regarding appropriate sample handling (see also section 1c) (41).

Table 1

Overview of CSF findings for $A\beta_{42}$, t-tau, and p-tau_{181} in the most common dementia disorders

Groups	$A\beta_{42}$	t-tau	p-tau_{181}
Normal ageing	N	N	N
Alzheimer's disease	↓↓	↑↑	↑↑
Dementia with Lewy bodies	↓	N / (↑)	N
Frontotemporal lobar degeneration	N / (↓)	variable	N / ↑
Vascular dementia	N / (↓)	N / ↑	N
Creutzfeldt-Jakob disease	↓	↑↑↑	N

CSF concentration: N = normal; ↑ = increased; ↓ = decreased.
$A\beta_{42}$ = amyloid β_{42} protein; t-tau = total tau; p-tau_{181} = tau phosphorylated at threonine 181.

P-tau, also measurable in CSF, appeared another promising biomarker in view of the neuropathological findings in AD. Tau can be phosphorylated at various sites, correlating with three stages of neurofibrillary tangle formation: 1) pre-neurofibrillary tangles (tau phosphorylated at threonine 231; p-tau_{231}); 2) intra-neuronal neurofibrillary tangles (tau phosphorylated at threonine 181; p-tau_{181}); and 3) extra-neuronal neurofibrillary tangles (tau phosphorylated at serine 199; p-tau_{199}) (46). Measurement of p-tau_{231} in CSF improved discrimination of AD from FTLD (47) (Table 1). and measurement of p-tau_{181} levels enhanced the discrimination of AD from DLB (48-50).

Subsequently, many studies followed that investigated all the above mentioned CSF markers in order to find the most optimal combination of markers for the discrimination between different dementias. The combination of CSF t-tau and $A\beta_{42}$ concentrations yielded a sensitivity varying between 81 and 94 percent and a specificity varying between 79 and 95 percent when differentiating between AD patients and normal controls (15;24;25;27;32), mostly established with the (imperfect) clinical diagnoses as the golden standard. However, the clinical challenge to distinguish AD from specific other dementia disorders still remained. The specificities for the distinction between AD and DLB, VaD, and FTD, were 67, 48, and 85 percent respectively (25;27). The $A\beta_{42}$/p-tau_{181} ratio was found to be a good discriminator between

AD and VaD (sensitivity, specificity, negative and positive predictive values all ≥ 85%) (51). AD and DLB could be differentiated by the t-tau/p-tau$_{181}$ ratio, but values still overlapped markedly (52). To facilitate simultaneous measurement of these three biomarkers, a new technique has been developed (see section 1d.).

CSF Aβ_{42}, t-tau, and p-tau analysis can also be useful in the discrimination between sporadic Creutzfeldt-Jakob disease (CJD) and other dementias. CJD is characterized by progressive dementia, pyramidal dysfunction, myoclonus, extrapyramidal signs, visual symptoms, and ataxia, but the differentiation from other dementia's, especially AD and FTLD, can be difficult very early in the course of CJD. In CSF of CJD patients, very high levels of t-tau were found. Using a cut-off value of 1300 pg/ml a diagnostic sensitivity of 94% with 90% specificity was achieved, with a positive predictive value of 92% (53). In another study a CSF t-tau cut-off value of 2131 pg/ml resulted in a sensitivity of 93% and specificity of 100% for the discrimination between CJD and AD (54). In accordance with the absence of hyperphosphorylation of tau proteins and NFTs in brains of CJD patients, relatively low CSF p-tau concentrations (< 85 ng/L) were found (55). Furthermore, the CSF p-tau/t-tau ratio was also found to be a good discriminator; CJD patients could be differentiated from AD and FTLD (55;56). CSF Aβ_{42} levels were decreased in CJD patients, but to a similar degree as in AD patients (54;57). The immunodetection of 14-3-3 proteins in CSF has also shown high sensitivity and specificity for CJD (53;58;59). However, further research revealed false negative results in some typical, autopsy proven CJD cases (59;60), and false positive results in patients with other dementias, including AD, DLB, FTD, and VaD (61;62). The concentrations of neuron-specific enolase (NSE) and S-100 protein are also elevated in CSF of CJD patients (63-66). Thus, CJD can be discriminated from other dementias by the combined analysis of Aβ_{42}, t-tau, p-tau$_{181}$, 14-3-3 protein, NSE and S-100 protein.

Postmortem verification studies

Although the neuropathological diagnosis of different dementias is considered the "golden standard", there are only a few studies that used autopsy-confirmed diagnoses as such. Two studies included only patients with autopsy-confirmed dementias. The first investigated the correlation of 106 antemortem CSF t-tau and Aβ_{42} levels with postmortem dementia diagnoses, and confirmed the earlier observations that

elevated t-tau levels and reduced $A\beta_{42}$ levels in CSF are associated with AD pathology, and can help discriminate AD from other dementias (37). Interestingly, there were also a few patients in this study who met the clinical and pathological criteria for AD, but had CSF t-tau levels below a pre-defined cutoff value. In the second and recently published study, levels of $A\beta_{42}$, t-tau and p-tau_{181} were determined in CSF samples from 100 autopsy-confirmed dementia and 100 control subjects. AD was optimally discriminated from non-AD dementias using p-tau_{181} and $A\beta_{42}$ (sensitivity 80%, specificity 93%) (67).

Other studies included a few autopsy confirmed patients within their larger group of dementia patients. Based on CSF levels of t-tau and $A\beta_{42}$, 12 out of 13 patients with postmortem diagnoses could be correctly classified as having AD or not in one study (15). In two other studies, a small number of autopsy confirmed dementia patients were included, but unfortunately mean CSF tau and $A\beta_{42}$ levels of these subgroups of AD and FTD patients were not described (17;40).

Thus, information about CSF biomarkers in autopsy confirmed AD patients is relatively limited, but generally confirms the observation in the more numerous studies based on clinical diagnosis of dementia syndromes. More studies will likely follow on this issue, also including more patients with autopsy confirmed dementia diagnoses other than AD. However, it is to be expected that the generally accepted view of CSF $A\beta_{42}$, t-tau and p-tau_{181} will not dramatically change with these additional studies. Ideally, we still need the conformation of the validation of CSF biomarkers against a randomly selected postmortem sample, in which referral and selection bias, possibly still important in the studies that have been performed up to now, has been excluded.

Effect of processing and storage conditions, and reference values

Several pre-analytical factors will have to be considered for correct and reliable analysis of $A\beta_{42}$, t-tau and p-tau_{181}. It was reported that sampling of CSF in tubes made of glass or polystyrene leads to up to 30% reduced concentrations of $A\beta_{42}$ relative to collection in polypropylene tubes (68). This was confirmed in one subsequent study (69) that showed that various Aβ peptides were up to 30% decreased in polystyrene tubes, the t-tau concentration decreased by 15%, while the p-tau_{181} concentration remained unaffected. Sampling in polycarbonate or modified polystyrene (polystyrene/acetonitrile mixture) did not affect results (69).

Prolonged storage of CSF at –80°C does not affect $A\beta_{42}$ or t-tau concentrations (70;71). CSF tau protein is even stable at up to 18°C for more than two weeks. $A\beta_{42}$ concentrations, however, may decrease by 20% during the first two days when stored at 4°C or 18°C while thereafter they remain stable (71). In contrast, in another study a small increase in the $A\beta_{42}$ concentration was demonstrated after storage for 24 hours at room temperature (72). We did not observe any effects on p-tau_{181} concentrations (unpublished data) when CSF was stored for 24 hours at –20°C, 4°C or 20°C. Neither did a single freeze/thaw cycle affect $A\beta_{42}$ or t-tau concentrations. $A\beta_{42}$ concentrations did decrease, however, by 20% after multiple freeze/thaw cycles, whereas t-tau concentrations remain stable (71). We did not observe any effects on p-tau_{181} concentrations after a single freeze-thaw cycle (unpublished data). Furthermore, blood contamination of CSF samples leads to reduced concentrations of t-tau, p-tau_{181} and $A\beta_{42}$ (own observations). Finally, we did not find any indication for a lumbar-ventricular gradient in the concentrations of t-tau, p-tau_{181} and $A\beta_{42}$. Recently, however, it was reported that the concentrations of Aβ may follow diurnal fluctuations (73), suggesting the need for standardized sampling procedures.

In summary, the following procedures are advised when analyzing t-tau, p-tau_{181} and $A\beta_{1\text{-}42}$ in CSF: 1) CSF should be collected and stored in polypropylene tubes. 2) For optimal analysis, CSF samples should be centrifuged, aliquoted and stored at –80°C as soon as possible after withdrawal, although the effects of short-term storage at higher temperatures on the analytical results are minimal. 3) Samples contaminated by blood or hemolytic CSF should not be used for analysis. 4) Repeated freeze/thawing of CSF should be avoided. 5) Aliquots from any of the CSF fractions that are collected can be used; there is no need for a standardization of the volume to be analyzed. 6) Because of possible diurnal fluctuations, CSF withdrawal should be performed at a standardized time point of the day, but the initial observations on this matter await confirmation.

Studies aimed at analyzing and comparing the performance of different laboratories using the same tests for analysis of $A\beta_{42}$, t-tau and p-tau_{181} have been rarely reported. In a study with 13 laboratories testing three CSF samples, coefficients of variation (CV) varied from 8.5% to 10.8% (p-tau_{181}), 8.5% to 30% (t-tau) and 20 to 60% ($A\beta_{42}$) for users of the Innogenetics assays (Innogenetics NV, Ghent, Belgium) (74). In a study with 14 participating laboratories testing a single CSF sample CV's varied

from 26 to 29% for these three assays (75). This implies that, as long as a uniform standardization of (pre-)analytical procedures has not been defined, inter-laboratory comparisons of test results are possible to a limited extent only. However, since intra-assay CV's are low (< 8.3% for all three assays as determined in our laboratory (76)), stable and reproducible results can be produced by one and the same laboratory over time. Finally, given the high inter-laboratory CV's, each laboratory should establish their own reference ranges.

The reference values for CSF t-tau and $A\beta_{42}$ levels using sandwich ELISAs by Innogenetics were established in 231 healthy individuals, 21–93 years of age, with a Mini-Mental State Examination score of 28 or above (77). Since a positive correlation was found between age and CSF t-tau, separate reference values for different age groups were established: < 300 ng/L (21–50 years), < 450 ng/L (51–70 years), and < 500 ng/L (71–93 years). The reference value for CSF-$A\beta_{42}$ was set to > 500 ng/L (Table 2). In addition, there was no correlation found between blood-CSF barrier (dys)function and levels of t-tau or $A\beta_{42}$ in CSF. In our own laboratory we established reference ranges with the Innogenetics assays that are largely in line with these data ($A\beta_{42}$: > 400 ng/L (< 15 years), > 500 ng/L (> 15 years); t-tau: < 300 ng/L (< 50 years), < 350 ng/L (> 50 years); p-tau_{181}: < 85 ng/L (> 15 years)) (Table 2). For reasons mentioned above, these reference ranges may not be universally applicable.

Table 2

Reference values for $A\beta_{42}$, t-tau, and p-tau_{181} in cerebrospinal fluid

CSF biomarker	Age (years)	Sjogren et al. (77) (ng/L)	Our laboratory (ng/L)
$A\beta_{42}$	< 15		> 400
	21 - 93	> 500	> 500
t-tau	21 - 50	< 300	< 300
	51 – 70	< 450	< 350
	71 - 93	< 500	< 350
p-tau_{181}	> 15		< 85

$A\beta_{42}$ = amyloid β_{42} protein; t-tau = total tau; p-tau_{181} = tau phosphorylated at threonine 181.

Multiplex analysis of $A\beta_{42}$, t-tau and p-tau_{181}

Robust analysis of $A\beta_{42}$, t-tau and p-tau_{181} should enhance their usefulness in AD diagnosis. As noted above, analysis of these three biomarkers by ELISA may lead to relatively high inter-laboratory variation. The recent development of assays based on the xMAP technology may possibly solve this issue. The Luminex xMAP technology has several advantages over conventional ELISA. It involves coupling of capture antibodies to microspheres (with a unique color code) and fluorescent dye-labeled detecting antibodies. By using a flow cytometry-based detector, multiple analytes can be detected simultaneously. Compared with ELISA, xMAP technology requires less total assay time, fewer procedural steps, and a smaller sample volume. It has a higher reproducibility than ELISA, because the result of each analysis is the mean of multiple (typical 50-100) readings. xMAP assays to quantify $A\beta_{42}$, t-tau, and p-tau_{181} in CSF, together with test characteristics for calibration, precision, and specificity have been recently described (78). Inter-assay CV's for the three analytes are all < 8.4 to 10% (76;78). In contrast to what has been suggested by others (78), we observed that a single correction factor cannot be applied to recalculate xMAP results into ELISA results (or vice versa), suggesting that the introduction of xMAP assays should be accompanied by a complete method validation, including a re-establishment of reference ranges specific for this assay which are different from those used for the ELISAs (76). However, both ELISA and xMAP assays had a comparable analytical performance to differentiate AD patients from either controls or VaD patients (76). In another recent, multi-center study, it was demonstrated that the xMAP assays for $A\beta_{42}$, t-tau, and p-tau_{181} may identify patients with mild cognitive impairment (MCI) that convert to AD among a population of MCI patients, just as has been described for the ELISA assays (79).

Relation with disease severity

In many studies, the correlation between disease severity and CSF levels of $A\beta_{42}$, t-tau, and p-tau was investigated. The results were not unanimous. Some studies did find a correlation between one or more CSF biomarkers and disease severity (13;15;80-82), while many others did not (14;29;30;83-86). Also, remarkably stable CSF $A\beta_{42}$, t-tau, and p-tau concentrations over a 6-month period were found in individual AD patients (87). In agreement with these latter findings, decreased CSF $A\beta_{42}$

levels and increased t-tau levels were already found very early in progression of AD, thus trading the correlation with disease progression for better diagnostic sensitivity. In line with this, in MCI patients, these biomarkers were able to predict progression to AD (88-92). Similarly, marked increases in CSF p-tau levels were found in MCI cases who at follow-up progressed to AD, compared to stable MCI cases (92;93). Similar results were achieved using the xMAP technology (94). The t-tau/$A\beta_{42}$ ratio or the p-tau_{181}/$A\beta_{42}$ ratio in CSF can also be used to predict conversion from cognitively normal to AD (95).

Since it is thought that Alzheimer pathology starts at least 20 to 30 years before the clinical onset of the disease (96), one could speculate that altered CSF levels of the above mentioned biomarkers are present even before cognitive dysfunction begins. Thus, when drugs with potential effects on the progression of AD reach the clinical phase, CSF diagnostics would be helpful in identifying people at risk of developing AD.

Discovery of novel biomarkers by proteomics

Technological advances over the past few years, and the recognition that it is indeed possible to define biomarkers in CSF for AD, have sparked the interest in proteomics techniques to identify such new biomarkers. Several approaches have been followed, either based on separate technologies, e.g hypothesis-free two-dimensional gel-electrophoresis (2D-GE) combined with matrix-assisted laser desorption/ionization time-of flight (MALDI-TOF), mass spectrometry (MS) or surface enhanced laser desorption/ionization (SELDI-) TOF MS, or application of these techniques to unbiased proteomics approaches (protein profiling without any type of preselection for biomarkers), or procedures with enrichment for specific protein species (e.g. SELDI-TOF with antibody capture).

Unbiased approaches; 2D-GE and MALDI-TOF analysis

Because of the extensive improvement in the techniques to perform 2D-GE and the increased possibilities to identify proteins by MS, the number of studies aimed at the identification of biomarkers in CSF by using this technique has grown steadily.

In 2D-GE proteins are separated in the first dimension on the basis of their pI by isoelectric focusing (IEF) and in the second dimension by SDS-PAGE according to their molecular weight. Specific protein spots can be picked out the gel, trypsin-digested and the resulting peptides are analyzed by (MALDI-TOF) MS or nano-LC-MS. Numerous technical papers have been published in which technical advances and methods have been described to pretreat or enrich CSF samples for low-abundant proteins. Several pre-treatment strategies (protein precipitation, column filtration, removal of albumin and immunoglobulins) may improve 2D-GE patterns (97), leading to large databases of CSF proteins (98). The use of liquid IEF (instead of solid-phase IEF) followed by SDS-PAGE and MS analysis allows for identification of proteins in the various fractions, but mainly high-abundant proteins are identified and large volumes of CSF are needed (99). An extra IEF step may resolve less abundant proteins (100). Irrespective the exact technology that has been applied, all the variants on the concept of 2D-GE have led to the definition of combinations of multiple proteins – ranging from 5 to 25 in number – that may discriminate AD from non-AD CSF by 2D-GE (see a recent review for a comprehensive overview (101)) (102-104). Most studies described alterations in abundant, mostly blood-derived proteins, that are often without any relation to the presumed pathophysiology of AD, and that are usually not confirmed by alternative techniques (e.g. ELISA) or in independent studies (105;106). One recent study, however, in which 2D-GE analysis of plasma proteins combined with LC-MS/MS characterization demonstrated increased concentrations of complement factor H and α2-macroglobulin in AD (107). However, although the findings from the 2D-GE studies indicated a large quantitative difference in the levels of these proteins, after confirmation by ELISA these differences appeared much smaller resulting in sensitivity, specificity and negative/positive predictive values all < 68%. Furthermore, the use of such combinations of protein markers relative to the analysis of $A\beta_{42}$, t-tau and p-tau_{181} has not yet been studied. Given the advances that have been achieved so far, it is unlikely that a laborious and relatively insensitive experimental approach such as 2D-GE combined with MS analysis, will ever reach clinical application, or lead to discovery of novel biomarkers for AD.

SELDI-TOF MS

SELDI-TOF is a technique that probably has more potential than 2D-GE/MS to identify novel biomarkers, since it allows not only for an unbiased approach in the search for biomarkers, but also for more specific identification of proteins e.g. by applying pre-selection with antibodies.

In a small study using CSF of 9 AD patients and 10 controls, four proteins (cystatin C, two β2-microglobulin isoforms, one unidentified protein) were over-expressed and one protein (VGF polypeptide) was under-expressed in AD CSF (108). In a somewhat larger study (16 FTLD and 12 controls) (109), five proteins were increased in FTLD and five others were decreased. Among the increased proteins were transthyretin and S-cysteinylated transthyretin, and among the decreased proteins were VGF, truncated cystatin C (amino acids 1-8 deleted) and a chromogranin B fragment. Finally, in a recent comparison with 65 AD and 44 control samples, a model with five proteins (decreased levels in AD: cystatin C and an unknown 4.0 kDa protein, increased levels in AD: truncated cystatin C, C3a anaphylatoxin des-Arg, $A\beta_{1\text{-}40}$) together with t-tau and $A\beta_{1\text{-}42}$ analysis optimally discriminated these groups (110). Also in this study, decreased concentrations of VGF were found. In general, relatively high-abundant proteins are selected in the SELDI procedures, that – in combination with t-tau and $A\beta_{1\text{-}42}$, may provide a useful panel of markers to discriminate either AD or FTLD from controls. Somewhat worryingly, several of the proteins identified in these studies and suggested as biomarkers for AD or FTLD (e.g. (truncated) cystatin C, VGF, transthyretin) have also been nominated as biomarkers for amyotrophic lateral sclerosis (111;112), schizophrenia (113), or multiple sclerosis (114), suggesting that several of the proteins (or combinations thereof) identified as biomarkers for a specific disease by SELDI-TOF, are more likely to be reflections of ongoing generalized neurodegeneration rather than related to specific pathophysiological disease processes. Alternatively, they may be selectively identified under the applied technical conditions. Obviously, larger validation studies with more clinical subgroups, and validation of the data by independent techniques, need to be performed to assess the clinical validity of these findings.

Aβ peptidomics identified by SELDI-TOF or gel electrophoresis

Most studies aimed at the identification of Aβ in CSF, have focused on $Aβ_{1-42}$ or $Aβ_{1-40}$. However, several other studies suggested the presence of numerous other Aβ species in the CSF, i.e. N-terminally or C-terminally truncated species, and Aβ peptides with an elongated C-terminus. Such peptides have been identified by SELDI-TOF or urea-based gel electrophoresis. Moreover, evidence is accumulating that dimeric or higher assembled forms of Aβ peptides may circulate in the CSF.

By coating the SELDI chip with an anti-Aβ capture antibody, it is possible to employ SELDI-TOF to specifically detect these alternative Aβ species present in the CSF. In a study with a small number of AD (n=10) and control patients (n=9), it was found that Aβ peptides other than $Aβ_{1-42}$ or $Aβ_{1-40}$ are present in CSF at abnormal concentrations in AD compared to controls. These new Aβ peptides could fit with the following sequences: $Aβ_{3-44}$ (decreased in AD), $Aβ_{3-47}$ (increased in AD) and a possible dimer of Aβ (decreased in AD) (115). Aβ peptides starting at residue 1 and ending at residues 37 to 40 were present in AD and control CSF at equal concentrations. In addition to these peptides, the same research group as well as another group published the identification of many different truncated Aβ peptides in the CSF (116;117), of which $Aβ_{1-38}$ was reported to be decreased in AD compared to controls in the one study (116), but not in the other (117). Recently, we performed a similar study with CSF of 18 AD, 20 VaD and 17 controls patients (unpublished observations). We observed decreased concentrations of $Aβ_{1-37}$, $Aβ_{1-38}$, $Aβ_{1-39}$ and $Aβ_{1-40}$ in AD compared to either VaD or controls. Furthermore, we found decreased levels of $Aβ_{1-33}$, $Aβ_{1-34}$, several oxidized Aβ peptides and possible dimeric and trimeric forms of Aβ in AD vs. VaD or controls. We uncovered preliminary evidence that a possible dimeric $Aβ_{1-38}$ peptide was decreased in both AD and VaD. These findings of altered concentrations of assembled forms of Aβ in CSF are in line with the observation that the concentrations of so-called Aβ-derived diffusible ligands ("oligomers") are altered in AD CSF compared to controls (118).

Urea-based gel electrophoresis allowed a good separation of Aβ peptides of various lengths (119). By using the $Aβ_{1-42}/Aβ_{1-37}$ ratio, AD patients (n=23) could be differentiated from DLB (n=21), idiopathic Parkinson's disease (n=21) or controls (n=23) (120). However, a disadvantage of this method may be that quantification of Aβ peptides is likely subject to larger analytical variation than, for example, specific

ELISAs. A combination of immunoprecipitation with anti-Aβ antibodies and subsequent MALDI-TOF yielded a significant increase in $A\beta_{1-16}$ in AD CSF and, in combination with $A\beta_{1-42}$, $A\beta_{1-33}$, and $A\beta_{1-39}$, may distinguish AD from control CSF (121).

The results of these various studies suggest that several of the identified Aβ peptides or aggregated forms of Aβ are worth to be further studied as a potential biomarker for AD, in particular by using specific quantification techniques (e.g ELISA), and in larger patient cohorts (also including other dementia forms).

Other potential CSF biomarkers

In the past years, numerous potential CSF biomarkers for AD and other dementias have been studied, e.g. amyloid precursor protein (APP), apolipoprotein E, α1-antichymotrypsin, C-reactive protein, complement C1q, homocysteine, 3-nitrotyrosine, neuronal thread protein, NSE, glial fibrillary acidic protein, S-100B protein, ubiquitin, and growth-associated protein-43 [for details, see other reviews (19;122-124)]. However, none of these proteins and metabolites appeared useful as biomarkers in clinical practice, because of conflicting results between different studies, or

Table 3

Potential CSF biomarkers for dementia

Group	Potential CSF biomarker	Reference no.
Isoprostanes	F2-isoprostane	(40, 125 – 127)
Cytokines	IL-1β	(123, 128)
	IL-6	(123, 128, 129)
	IL-12	(130)
	IL-15	(131)
	TNF-alpha	(123, 128)
Neurofilaments	NFL	(132 – 138)
	NFH/M	(132)
	NFH	(138, 139)
Aβ oligomers		(118, 140 – 142)
Enzymes	BACE-1	(143)
	ACE	(144, 145)

because of an inferior capacity to discriminate between different dementias compared to $A\beta_{42}$, t-tau or p-tau_{181}. More recently studied potential CSF biomarkers are described below (see also table 3).

Isoprostanes

Oxidative damage may play a role in the pathogenesis of AD. Isoprostanes are exclusive products of free-radical-mediated peroxidation of arachidonic acid. Levels of isoprostanes were found markedly elevated in both frontal and temporal cortex of AD brains, but not in the corresponding areas of FTLD brains and controls (125;126). F2-isoprostanes are the most studied species, and their CSF concentrations can be measured by gas chromatography/negative ion chemical ionization MS. CSF F2-isoprostanes were found significantly increased in patients with probable AD, compared with controls (40;127;128), and compared to FTLD patients (40). Levels were highly correlated with disease severity (127;128). Furthermore, in AD patients and patients with non-AD dementias the combined analysis of CSF F2-isoprostane levels, $A\beta_{42}$ and t-tau levels resulted in sensitivity of 84% and specificity of 89% (129). Others performed a discriminant analysis based on CSF levels of t-tau, $A\beta_{42}$, and F2-isoprostane and were able to classify 88.5% of patients in a manner that corresponded to their clinical or autopsy diagnosis (40). F2-isoprostane levels were also increased in CSF of subjects with MCI, compared to cognitively normal elderly subjects (130). This implies that brain oxidative damage starts early in the course of AD, and that F2-isoprostanes can be used as CSF markers before the onset of symptomatic dementia. Additional studies, including confirmation by independent research groups, are warranted, however, to establish F2-isoprostane analysis as a biomarker for AD.

Cytokines

In AD brains, the pathological lesions, in particular senile plaques, are associated with a localized neuro-inflammatory reaction, characterized by activated microglia and astrocytes, with increased expression of inflammatory proteins such as cytokines (131). Several cytokines can be detected in CSF, but the results of CSF cytokine measurements in dementia patients are still controversial (reviewed by Teunissen et al.) (123). In AD patients IL-6 was found increased, decreased, or not significantly

different from controls in different studies (123;132). CSF concentrations of IL-6 were also significantly elevated in VaD patients (132;133). Differentiation of AD and VaD by measuring CSF IL-6 levels was possible in one study (133), that could not be confirmed by others (132). The same applies for IL-1β and TNF-alpha. Some studies found increased CSF levels in AD patients while others did not (123;132). CSF levels of TNF-alpha are also increased in VaD patients (132). In the CSF of FTLD patients IL-15 was elevated, and IL-12 levels were reduced (134;135), but the same phenomenon was found in AD patients (134;135).

Thus, although CSF levels of cytokines in dementia patients appear different from controls, their quantifications can not yet be used as biomarkers to differentiate between different dementia disorders. In the future, however, CSF cytokines maintain the potential to provide assistance in monitoring treatment effects of neuroprotective drugs, especially anti-inflammatory drugs.

Neurofilaments

Neurofilament proteins (NFs) are the major constituents of the neuronal cytoskeleton. They are predominantly localized in large neurons and myelinated axons, and play an important role in neuronal structure and function. NFs consist of three polypeptides with different molecular weights, known as the light (NFL), medium (NFM) and heavy (NFH) subunits, respectively (136). Since NFs are released from damaged neurons, increased levels of NF protein in CSF may reflect the degree of neuronal degeneration in neurological disease.

Since 1996, the analyses of NF proteins in CSF of patients with different neurodegenerative disorders, including dementia, challenged various researchers. NFL levels in CSF were found to be increased in AD and FTLD, compared to control subjects (137-143). More specifically, CSF NFL levels were elevated in relatively young patients with FTLD compared to patients with early-onset AD (141;143). When combining CSF levels of NFL with p-tau$_{181}$ and Aβ_{42}, differentiating between FTLD and early-onset AD was possible with a sensitivity of 86%, and specificity of 100% (143).

In contrast to studies on NFL, less is known about the CSF levels of the other two NF subunits in neurodegenerative dementias. Higher CSF levels of phosphorylated NFH/M were found in AD patients compared to VaD patients and controls (137).

CSF levels of NFH were increased in patients with AD, VaD, FTLD, and DLB, compared to control subjects (143;144). However, discriminating between different dementia disorders using CSF NFH levels was only possible in DLB versus early-onset AD (143).

Although increased CSF levels of NFL and NFH were found in different dementia disorders, their analysis showed no superior, and only limited additional, diagnostic value compared to the frequently used CSF biomarkers $A\beta_{42}$, t-tau and p-tau_{181} (143).

Aβ oligomers

Results from the above-mentioned studies using the SELDI-TOF technology suggest the occurrence of Aβ aggregates, particularly dimers, trimers and possibly larger species, in CSF of AD patients. However, the quantification and definite identification of such Aβ oligomers cannot be achieved by SELDI-TOF, and more specific assays are needed for this goal. Already back in 1998, a study was published in which the presence at increased concentrations of Aβ oligomers in CSF was demonstrated by seeded polymerization of labeled Aβ onto Aβ oligomeric seeds, visualized by fluorescence correlation spectroscopy (145). More recently, identification of Aβ oligomers in CSF was achieved by a combination of protein immunodetection and DNA amplification techniques in the so-called bio-barcode assay (118). Aβ oligomer concentrations were increased in AD compared to controls. Advanced Aβ oligomerization may lead to an underestimation of Aβ levels, since the commonly used assays preferentially detect Aβ monomers (146). Recently, Aβ oligomers were detected in CSF by using flow cytometry and fluorescence resonance energy transfer (147). Although such oligomers appear to be promising new biomarkers for AD, more validation studies with large clinically well-defined subgroups of dementia syndromes are needed to establish their potential use as a biomarker. Furthermore, more extensive analysis and characterization of the type of Aβ aggregates that are detected by the respective assays are needed to assess the robustness of such assays.

Enzymes

Various enzymes in CSF have been studied as potential biomarkers for AD, but only few are promising. The beta-site APP cleaving enzyme 1 (BACE-1) is responsible for the first proteolytic event in the cleavage of APP leading to amyloid formation.

Inhibition of BACE-1 is thought to be a therapeutic approach to AD. CSF BACE-1 activity was found elevated in AD patients in comparison with a group of patients with other dementias (148). Thus, detection of BACE-1 in CSF may have diagnostic applications, and may also be useful for monitoring the effects of drugs. Further research is needed.

Another potentially useful enzyme is the angiotensin converting enzyme (ACE). There is increasing evidence that ACE plays a role in the development of AD. It has been suggested that decreased ACE activity may influence the susceptibility to AD by a mechanism involving Aβ metabolism (149). However, no difference in CSF ACE levels was found between AD, DLB and controls (150). Others found elevated CSF ACE activity in AD-converted MCI patients and mild to moderate AD patients as compared to controls (151). A brain-penetrating ACE-inhibitor was able to significantly inhibit CSF ACE activity in AD patients (151). Thus, a role for ACE as a possible marker for disease and drug effects remains a possibility.

Plasma biomarkers for AD

Since blood is easier accessible than cerebrospinal fluid, the search is on for useful plasma biomarkers, especially $A\beta_{40}$ and $A\beta_{42}$. Although there was no relation between plasma $A\beta_{40}$ and $A\beta_{42}$ levels and those in CSF (152), or between plasma Aβ levels and brain Aβ levels (153), some groups reported increased plasma $A\beta_{42}$ levels in AD patients or MCI patients that converted to AD (154-156). Plasma $A\beta_{40}$ levels were not increased in these patients (154;157). Similar to biomarkers in CSF, an increased ratio of plasma $A\beta_{42}$ and $A\beta_{40}$ levels correlated with a decrease in dementia risk (158), and vice versa (159). In most studies, plasma $A\beta_{42}$ levels were positively correlated with age (154;157), but not with cognitive impairment or duration of the disease (152;157). Thus, when interpreting the results of plasma Aβ levels, the effect of age should be taken into account. Also, the consequence of concomitant medication should be considered. Increased levels of plasma $A\beta_{42}$ were found in non-demented subjects who used insulin and biguanides, decreased levels in users of gingko biloba and statin users, and a nonsignificant trend to reduced levels was found in users of nonsteroidal anti-inflammatory drugs (NSAIDs) (160). Although another study could

not confirm the altered plasma $A\beta_{42}$ levels in statin and NSAID users (157), these findings will complicate the interpretation of the results.

In conclusion, measurement of plasma $A\beta_{42}$ levels or the plasma $A\beta_{42}/A\beta_{40}$ ratio is currently not useful for the diagnosis of AD, but it may be interpreted as a biological risk factor. Changes related to specific medication should be taken into account.

What is the future of biomarkers for AD?

Still the most successful neurochemical biomarkers for AD are the analysis in CSF of $A\beta_{42}$, t-tau, and p-tau_{181}. However, CSF analysis of t-tau, p-tau and $A\beta_{42}$ also has some limitations. Although the test characteristics are excellent to differentiate AD from controls, the real challenge is in the differentiation of AD from other dementia disorders, that commonly include prion disease (e.g. CJD), VaD, tauopathies (e.g. FTLD, progressive supranuclear palsy) and α-synucleinopathies (DLB, Parkinson's disease). CJD can be identified by CSF analysis of, particularly, 14-3-3 and t-tau proteins with very high sensitivity and specificity. However, only a very small proportion of dementia patients suffers from CJD (incidence 1-1.5: 1,000,000 people). Furthermore, we demonstrated that AD can be very well differentiated from VaD by calculating the $A\beta_{42}$/p-tau ratio, which accurately separates both disorders (sensitivity, specificity and negative /positive predictive values> 85%) (51). However, the differentiation of AD from other dementia syndromes remains a challenge and is not optimal by using these CSF biomarkers. Basically this is caused by low $A\beta_{42}$ levels in DLB and highly variable t-tau and p-tau levels in FTLD. Therefore, the application of CSF testing seems to be particularly restricted to the identification of AD patients amongst a population of dementia patients.

Essentially, almost all studies aimed at the estimation of the diagnostic accuracy of CSF analysis in dementia syndromes have been carried out in specific and selected patient cohorts as part of retrospective case-control studies, in expert referral clinics, and usually not based on predetermined cut-off values ("phase I and IIa,b") (161). Phase III studies, in which one should use the test with predetermined cut-off values to discriminate between cases and non-cases, have been attempted,

but mostly failed because of insufficient blinding of the clinicians for the CSF measurements in the process of defining a diagnosis and in the omission of strictly predefined cut-off values. Extension of the studies referred here with a "phase III" clinical effectiveness study, in which the additional value of CSF analysis will be investigated in a cohort of patients visiting memory clinics, will be an essential step forward in the evaluation of the validity of CSF testing in the diagnostic pathway for AD as it appears in daily practice.

A recent study described how new diagnostic tests can be categorized and tested against existing diagnostic pathways (162). Three different types of new diagnostic tests can be defined: 1) Replacement tests. The introduction of a new test is superior to existing diagnostic test and entirely replaces the old ones; 2) Triage tests. Triage tests may be less accurate than existing ones, but may be performed prior to the existing tests as a first line of rapid screening; 3) Add-on tests. This type of tests does not replace existing tests and is performed after these existing tests in a specific subgroup of patients. Typically, CSF analysis of t-tau, p-tau and $A\beta_{42}$ should be regarded as "add-on" tests in the diagnostic pathways, since in cases of an unequivocal clinical diagnosis of AD or non-AD, additional diagnostic investigations including CSF analysis, are not indicated. We have recently started a diagnostic phase III study in a population of patients visiting a memory clinic for evaluation of their dementia syndrome, in which the diagnostic value of CSF analysis of $A\beta_{42}$, t-tau, and p-tau_{181} will be evaluated in an add-on design.

In conclusion, CSF analysis of t-tau, p-tau and $A\beta_{42}$ is still the standard in the neurochemical diagnosis of AD. Novel biomarkers for AD are extensively searched for by several research groups. Novel technologies, including MS analysis, may lead to the discovery of new biomarkers. Probably the most promising type of new biomarkers are still to be found in CSF. However, if new techniques that allow for the sensitive detection of very low levels of brain-specific proteins are being developed, it is most feasible that biomarker analysis in CSF will be replaced by the more easily accessible blood. The presence of assembled forms of Aβ (dimers, trimers, oligomers) may serve as potential biomarkers for AD. In addition, N- or C-terminally truncated Aβ peptides may also add to the diagnostic specificity of CSF analysis for AD. Specific and robust tests to detect this variety of Aβ (-derived) peptides and aggregates need to be developed, however. Furthermore, the analysis of isoprostanes, and possibly

other specific metabolites related to the oxidative process in AD, may serve as a biomarker. Confirming studies by other groups should be performed, however. For all the newly described potential biomarkers extensive phase I and II studies will have to be performed, however, before they can be evaluated in clinical practice.

References

(1) Arnold SE, Hyman BT, Flory J, Damasio AR, Van Hoesen GW. The topographical and neuroanatomical distribution of neurofibrillary tangles and neuritic plaques in the cerebral cortex of patients with Alzheimer's disease. Cereb Cortex 1991 Jan;1(1):103-116.

(2) Braak H, Braak E. Neuropathological stageing of Alzheimer-related changes. Acta Neuropathol (Berl) 1991;82(4):239-259.

(3) McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 1984 Jul;34(7):939-944.

(4) Clinical and neuropathological criteria for frontotemporal dementia. The Lund and Manchester Groups. J Neurol Neurosurg Psychiatry 1994 Apr;57(4):416-418.

(5) McKeith IG, Galasko D, Kosaka K, et al. Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. Neurology 1996 Nov;47(5):1113-1124.

(6) Roman GC, Tatemichi TK, Erkinjuntti T, et al. Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993 Feb;43(2):250-260.

(7) Nagy Z, Esiri MM, Hindley NJ, et al. Accuracy of clinical operational diagnostic criteria for Alzheimer's disease in relation to different pathological diagnostic protocols. Dement Geriatr Cogn Disord 1998 Jul;9(4):219-226.

(8) Varma AR, Snowden JS, Lloyd JJ, Talbot PR, Mann DM, Neary D. Evaluation of the NINCDS-ADRDA criteria in the differentiation of Alzheimer's disease and frontotemporal dementia. J Neurol Neurosurg Psychiatry 1999 Feb;66(2):184-188.

(9) Lopez OL, Litvan I, Catt KE, et al. Accuracy of four clinical diagnostic criteria for the diagnosis of neurodegenerative dementias. Neurology 1999 Oct 12;53(6):1292-1299.

(10) Luis CA, Barker WW, Gajaraj K, et al. Sensitivity and specificity of three clinical criteria for

dementia with Lewy bodies in an autopsy-verified sample. Int J Geriatr Psychiatry 1999 Jul;14(7):526-533.

(11) Verghese J, Crystal HA, Dickson DW, Lipton RB. Validity of clinical criteria for the diagnosis of dementia with Lewy bodies. Neurology 1999 Dec 10;53(9):1974-1982.

(12) Gold G, Bouras C, Canuto A, et al. Clinicopathological validation study of four sets of clinical criteria for vascular dementia. Am J Psychiatry 2002 Jan;159(1):82-87.

(13) Tato RE, Frank A, Hernanz A. Tau protein concentrations in cerebrospinal fluid of patients with dementia of the Alzheimer type. J Neurol Neurosurg Psychiatry 1995 Sep;59(3):280-283.

(14) Blennow K, Wallin A, Agren H, Spenger C, Siegfried J, Vanmechelen E. Tau protein in cerebrospinal fluid: a biochemical marker for axonal degeneration in Alzheimer disease? Mol Chem Neuropathol 1995 Dec;26(3):231-245.

(15) Galasko D, Chang L, Motter R, et al. High cerebrospinal fluid tau and low amyloid beta42 levels in the clinical diagnosis of Alzheimer disease and relation to apolipoprotein E genotype. Arch Neurol 1998 Jul;55(7):937-945.

(16) Galasko D. Cerebrospinal fluid levels of A beta 42 and tau: potential markers of Alzheimer's disease. J Neural Transm Suppl 1998;53:209-221.

(17) Sunderland T, Linker G, Mirza N, et al. Decreased beta-amyloid1-42 and increased tau levels in cerebrospinal fluid of patients with Alzheimer disease. JAMA 2003 Apr 23;289(16):2094-2103.

(18) Blennow K, Hampel H. CSF markers for incipient Alzheimer's disease. Lancet Neurol 2003 Oct;2(10):605-613.

(19) Verbeek MM, De Jong D, Kremer HP. Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases. Ann Clin Biochem 2003 Jan;40(Pt 1):25-40.

(20) Sjogren M, Andreasen N, Blennow K. Advances in the detection of Alzheimer's disease-use of cerebrospinal fluid biomarkers. Clin Chim Acta 2003 Jun;332(1-2):1-10.

(21) Tamaoka A, Sawamura N, Fukushima T, et al. Amyloid beta protein 42(43) in cerebrospinal fluid of patients with Alzheimer's disease. J Neurol Sci 1997 May 1;148(1):41-45.

(22) Andreasen N, Hesse C, Davidsson P, et al. Cerebrospinal fluid beta-amyloid(1-42) in Alzheimer disease: differences between early- and late-onset Alzheimer disease and stability during the course of disease. Arch Neurol 1999 Jun;56(6):673-680.

(23) Shoji M, Matsubara E, Kanai M, et al. Combination assay of CSF tau, A beta 1-40 and A beta 1-42(43) as a biochemical marker of Alzheimer's disease. J Neurol Sci 1998 Jun 30;158(2):134-140.

(24) Hulstaert F, Blennow K, Ivanoiu A, et al. Improved discrimination of AD patients using beta-amyloid(1-42) and tau levels in CSF. Neurology 1999 May 12;52(8):1555-1562.

(25) Andreasen N, Minthon L, Davidsson P, et al. Evaluation of CSF-tau and CSF-Abeta42 as diagnostic markers for Alzheimer disease in clinical practice. Arch Neurol 2001 Mar;58(3):373-379.

(26) Kanemaru K, Kameda N, Yamanouchi H. Decreased CSF amyloid beta42 and normal tau levels in dementia with Lewy bodies. Neurology 2000 May 9;54(9):1875-1876.

(27) Riemenschneider M, Wagenpfeil S, Diehl J, et al. Tau and Abeta42 protein in CSF of patients with frontotemporal degeneration. Neurology 2002 Jun 11;58(11):1622-1628.

(28) Tapiola T, Overmyer M, Lehtovirta M, et al. The level of cerebrospinal fluid tau correlates with neurofibrillary tangles in Alzheimer's disease. Neuroreport 1997 Dec 22;8(18):3961-3963.

(29) Andreasen N, Vanmechelen E, Van d, V, et al. Cerebrospinal fluid tau protein as a biochemical marker for Alzheimer's disease: a community based follow up study. J Neurol Neurosurg Psychiatry 1998 Mar;64(3):298-305.

(30) Mecocci P, Cherubini A, Bregnocchi M, et al. Tau protein in cerebrospinal fluid: a new diagnostic and prognostic marker in Alzheimer disease? Alzheimer Dis Assoc Disord 1998 Sep;12(3):211-214.

(31) Shoji M, Matsubara E, Murakami T, et al. Cerebrospinal fluid tau in dementia disorders: a large scale multicenter study by a Japanese study group. Neurobiol Aging 2002 May;23(3):363-370.

(32) Gomez-Tortosa E, Gonzalo I, Fanjul S, et al. Cerebrospinal fluid markers in dementia with lewy bodies compared with Alzheimer disease. Arch Neurol 2003 Sep;60(9):1218-1222.

(33) Arai H, Satoh-Nakagawa T, Higuchi M, et al. No increase in cerebrospinal fluid tau protein levels in patients with vascular dementia. Neurosci Lett 1998 Nov 13;256(3):174-176.

(34) Green AJ, Harvey RJ, Thompson EJ, Rossor MN. Increased tau in the cerebrospinal fluid of patients with frontotemporal dementia and Alzheimer's disease. Neurosci Lett 1999 Jan 8;259(2):133-135.

(35) Kahle PJ, Jakowec M, Teipel SJ, et al. Combined assessment of tau and neuronal thread protein in Alzheimer's disease CSF. Neurology 2000 Apr 11;54(7):1498-1504.

(36) Itoh N, Arai H, Urakami K, et al. Large-scale, multicenter study of cerebrospinal fluid tau protein phosphorylated at serine 199 for the antemortem diagnosis of Alzheimer's disease. Ann Neurol 2001 Aug;50(2):150-156.

(37) Clark CM, Xie S, Chittams J, et al. Cerebrospinal fluid tau and beta-amyloid: how well do these biomarkers reflect autopsy-confirmed dementia diagnoses? Arch Neurol 2003 Dec;60(12):1696-1702.

(38) Pijnenburg YA, Schoonenboom NS, Rosso SM, et al. CSF tau and Abeta42 are not useful in the diagnosis of frontotemporal lobar degeneration. Neurology 2004 May

11;62(9):1649.

(39) Alberici A, Armani M, Paterlini A, et al. Tau missing from CSF: A case report. J Neurol 2007 Feb 3;254:107-109.

(40) Grossman M, Farmer J, Leight S, et al. Cerebrospinal fluid profile in frontotemporal dementia and Alzheimer's disease. Ann Neurol 2005 May;57(5):721-729.

(41) Verbeek MM, Pijnenburg YA, Schoonenboom NS, Kremer BP, Scheltens P. Cerebrospinal fluid tau levels in frontotemporal dementia. Ann Neurol 2005 Oct;58(4):656-657.

(42) Bergmann M, Kuchelmeister K, Schmid KW, Kretzschmar HA, Schroder R. Different variants of frontotemporal dementia: a neuropathological and immunohistochemical study. Acta Neuropathol (Berl) 1996 Aug;92(2):170-179.

(43) Spillantini MG, Crowther RA, Kamphorst W, Heutink P, van Swieten JC. Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. Am J Pathol 1998 Nov;153(5):1359-1363.

(44) Baker M, Mackenzie IR, Pickering-Brown SM, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 2006 Aug 24;442(7105):916-919.

(45) Cruts M, Gijselinck I, van der ZJ, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature 2006 Aug 24;442(7105):920-924.

(46) Augustinack JC, Schneider A, Mandelkow EM, Hyman BT. Specific tau phosphorylation sites correlate with severity of neuronal cytopathology in Alzheimer's disease. Acta Neuropathol (Berl) 2002 Jan;103(1):26-35.

(47) Buerger K, Zinkowski R, Teipel SJ, et al. Differential diagnosis of Alzheimer disease with cerebrospinal fluid levels of tau protein phosphorylated at threonine 231. Arch Neurol 2002 Aug;59(8):1267-1272.

(48) Parnetti L, Lanari A, Amici S, Gallai V, Vanmechelen E, Hulstaert F. CSF phosphorylated tau is a possible marker for discriminating Alzheimer's disease from dementia with Lewy bodies. Phospho-Tau International Study Group. Neurol Sci 2001 Feb;22(1):77-78.

(49) Hampel H, Buerger K, Zinkowski R, et al. Measurement of phosphorylated tau epitopes in the differential diagnosis of Alzheimer disease: a comparative cerebrospinal fluid study. Arch Gen Psychiatry 2004 Jan;61(1):95-102.

(50) Vanderstichele H, De VK, Blennow K, et al. Analytical performance and clinical utility of the INNOTEST PHOSPHO-TAU181P assay for discrimination between Alzheimer's disease and dementia with Lewy bodies. Clin Chem Lab Med 2006;44(12):1472-1480.

(51) De Jong D, Jansen RWMM, Kremer HPH, Verbeek MM. The cerebrospinal fluid amyloid β42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular

dementia. J Gerontol A Biol Sci Med Sci 2006;61(7):755-758.

(52) Mollenhauer B, Bibl M, Wiltfang J, et al. Total tau protein, phosphorylated tau (181p) protein, beta-amyloid(1-42), and beta-amyloid(1-40) in cerebrospinal fluid of patients with dementia with Lewy bodies. Clin Chem Lab Med 2006;44(2):192-195.

(53) Otto M, Wiltfang J, Cepek L, et al. Tau protein and 14-3-3 protein in the differential diagnosis of Creutzfeldt-Jakob disease. Neurology 2002 Jan 22;58(2):192-197.

(54) Kapaki E, Kilidireas K, Paraskevas GP, Michalopoulou M, Patsouris E. Highly increased CSF tau protein and decreased beta-amyloid (1-42) in sporadic CJD: a discrimination from Alzheimer's disease? J Neurol Neurosurg Psychiatry 2001 Sep;71(3):401-403.

(55) Buerger K, Otto M, Teipel SJ, et al. Dissociation between CSF total tau and tau protein phosphorylated at threonine 231 in Creutzfeldt-Jakob disease. Neurobiol Aging 2006 Jan;27(1):10-15.

(56) Riemenschneider M, Wagenpfeil S, Vanderstichele H, et al. Phospho-tau/total tau ratio in cerebrospinal fluid discriminates Creutzfeldt-Jakob disease from other dementias. Mol Psychiatry 2003 Mar;8(3):343-347.

(57) Otto M, Esselmann H, Schulz-Shaeffer W, et al. Decreased beta-amyloid1-42 in cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. Neurology 2000 Mar 14;54(5):1099-1102.

(58) Lemstra AW, van Meegen MT, Vreyling JP, et al. 14-3-3 testing in diagnosing Creutzfeldt-Jakob disease: a prospective study in 112 patients. Neurology 2000 Aug 22;55(4):514-516.

(59) Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. N Engl J Med 1996 Sep 26;335(13):924-930.

(60) Zerr I, Pocchiari M, Collins S, et al. Analysis of EEG and CSF 14-3-3 proteins as aids to the diagnosis of Creutzfeldt-Jakob disease. Neurology 2000 Sep 26;55(6):811-815.

(61) Burkhard PR, Sanchez JC, Landis T, Hochstrasser DF. CSF detection of the 14-3-3 protein in unselected patients with dementia. Neurology 2001 Jun 12;56(11):1528-1533.

(62) Huang N, Marie SK, Livramento JA, Chammas R, Nitrini R. 14-3-3 protein in the CSF of patients with rapidly progressive dementia. Neurology 2003 Aug 12;61(3):354-357.

(63) Jimi T, Wakayama Y, Shibuya S, et al. High levels of nervous system-specific proteins in cerebrospinal fluid in patients with early stage Creutzfeldt-Jakob disease. Clin Chim Acta 1992 Oct 15;211(1-2):37-46.

(64) Zerr I, Bodemer M, Racker S, et al. Cerebrospinal fluid concentration of neuron-specific enolase in diagnosis of Creutzfeldt-Jakob disease. Lancet 1995 Jun 24;345(8965):1609-1610.

(65) Kropp S, Zerr I, Schulz-Schaeffer WJ, et al. Increase of neuron-specific enolase in patients with Creutzfeldt-Jakob disease. Neurosci Lett 1999 Feb 12;261(1-2):124-126.

(66) Otto M, Stein H, Szudra A, et al. S-100 protein concentration in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease. J Neurol 1997 Sep;244(9):566-570.

(67) Engelborghs S, De Vreese K, Van de Casteele T, et al. Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. Neurobiol Aging 2007 Apr 9;doi:10.1016/j.neurobiolaging.2007.02.016.

(68) Vanderstichele H, Blennow K, D'Heuvaert N, et al. Development of a specific diagnostic test for the measurement of beta-amyloid(1-42) in CSF. In: Fisher A, Hanin I, Yoshida M, eds. Progress in Alzheimer's and Parkinson's diseases. New York: Plenum Publishing Group, 1998:773-778.

(69) Lewczuk P, Beck G, Esselmann H, et al. Effect of sample collection tubes on cerebrospinal fluid concentrations of tau proteins and amyloid beta peptides. Clin Chem 2006 Feb;52(2):332-334.

(70) Wilkinson D, Murray J. Galantamine: a randomized, double-blind, dose comparison in patients with Alzheimer's disease. Int J Geriatr Psychiatry 2001 Sep;16(9):852-857.

(71) Schoonenboom NS, Mulder C, Vanderstichele H, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implications for use in clinical practice. Clin Chem 2005 Jan;51(1):189-195.

(72) Kaiser E, Schonknecht P, Thomann PA, Hunt A, Schroder J. Influence of delayed CSF storage on concentrations of phospho-tau protein (181), total tau protein and beta-amyloid (1-42). Neurosci Lett 2007 May 1;417(2):193-195.

(73) Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007 Feb 27;68(9):666-669.

(74) Blankenstein MA, Clark CM, De Deyn PP, et al. Multicenter comparison of assays for cerebrospinal fluid (CSF) markers for Alzheimer's disease. Clin Chim Acta 2005;355S:S175.

(75) Lewczuk P, Beck G, Ganslandt O, et al. International quality control survey of neurochemical dementia diagnostics. Neurosci Lett 2006 Nov 27;409(1):1-4.

(76) Reijn TS, Olde Rikkert M, Van Geel WJ, De Jong D, Verbeek MM. Diagnostic Accuracy of ELISA and xMAP Technology for Analysis of Amyloid {beta}42 and Tau Proteins. Clin Chem 2007 May;53(5):859-865.

(77) Sjogren M, Vanderstichele H, Agren H, et al. Tau and Abeta42 in cerebrospinal fluid from healthy adults 21-93 years of age: establishment of reference values. Clin Chem 2001 Oct;47(10):1776-1781.

(78) Olsson A, Vanderstichele H, Andreasen N, et al. Simultaneous measurement of beta-

amyloid(1-42), total tau, and phosphorylated tau (Thr181) in cerebrospinal fluid by the xMAP technology. Clin Chem 2005 Feb;51(2):336-345.

(79) Lewczuk P, Kornhuber J, Vanderstichele H, et al. Multiplexed quantification of dementia biomarkers in the CSF of patients with early dementias and MCI: A multicenter study. Neurobiol Aging 2007 Jan 18;doi:10.1016/j.neurobiolaging.2006.12.010.

(80) Hock C, Golombowski S, Naser W, Muller-Spahn F. Increased levels of tau protein in cerebrospinal fluid of patients with Alzheimer's disease--correlation with degree of cognitive impairment. Ann Neurol 1995 Mar;37(3):414-415.

(81) Csernansky JG, Miller JP, McKeel D, Morris JC. Relationships among cerebrospinal fluid biomarkers in dementia of the Alzheimer type. Alzheimer Dis Assoc Disord 2002 Jul;16(3):144-149.

(82) Wallin AK, Blennow K, Andreasen N, Minthon L. CSF biomarkers for Alzheimer's Disease: levels of beta-amyloid, tau, phosphorylated tau relate to clinical symptoms and survival. Dement Geriatr Cogn Disord 2006;21(3):131-138.

(83) Arai H, Terajima M, Miura M, et al. Tau in cerebrospinal fluid: a potential diagnostic marker in Alzheimer's disease. Ann Neurol 1995 Oct;38(4):649-652.

(84) Vigo-Pelfrey C, Seubert P, Barbour R, et al. Elevation of microtubule-associated protein tau in the cerebrospinal fluid of patients with Alzheimer's disease. Neurology 1995 Apr;45(4):788-793.

(85) Riemenschneider M, Buch K, Schmolke M, Kurz A, Guder WG. Cerebrospinal protein tau is elevated in early Alzheimer's disease. Neurosci Lett 1996 Jul 19;212(3):209-211.

(86) Stefani A, Martorana A, Bernardini S, et al. CSF markers in Alzheimer disease patients are not related to the different degree of cognitive impairment. J Neurol Sci 2006 Dec 21;251(1-2):124-128.

(87) Blennow K, Zetterberg H, Minthon L, et al. Longitudinal stability of CSF biomarkers in Alzheimer's disease. Neurosci Lett 2007 May 23;419(1):18-22.

(88) Ivanoiu A, Sindic CJ. Cerebrospinal fluid TAU protein and amyloid beta42 in mild cognitive impairment: prediction of progression to Alzheimer's disease and correlation with the neuropsychological examination. Neurocase 2005 Feb;11(1):32-39.

(89) Bouwman FH, Schoonenboom SN, van der Flier WM, et al. CSF biomarkers and medial temporal lobe atrophy predict dementia in mild cognitive impairment. Neurobiol Aging 2006 Jun 16;28:1070-1074.

(90) Hampel H, Teipel SJ, Fuchsberger T, et al. Value of CSF beta-amyloid1-42 and tau as predictors of Alzheimer's disease in patients with mild cognitive impairment. Mol Psychiatry 2004 Jul;9(7):705-710.

(91) Andreasen N, Minthon L, Vanmechelen E, et al. Cerebrospinal fluid tau and Abeta42 as

predictors of development of Alzheimer's disease in patients with mild cognitive impairment. Neurosci Lett 1999 Sep 24;273(1):5-8.

(92) Herukka SK, Helisalmi S, Hallikainen M, Tervo S, Soininen H, Pirttila T. CSF Abeta42, Tau and phosphorylated Tau, APOE epsilon4 allele and MCI type in progressive MCI. Neurobiol Aging 2007 Apr;28(4):507-514.

(93) Buerger K, Teipel SJ, Zinkowski R, et al. CSF tau protein phosphorylated at threonine 231 correlates with cognitive decline in MCI subjects. Neurology 2002 Aug 27;59(4):627-629.

(94) Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006 Mar;5(3):228-234.

(95) Fagan AM, Roe CM, Xiong C, Mintun MA, Morris JC, Holtzman DM. Cerebrospinal fluid tau/beta-amyloid(42) ratio as a prediction of cognitive decline in nondemented older adults. Arch Neurol 2007 Mar;64(3):343-349.

(96) Price JL, Morris JC. Tangles and plaques in nondemented aging and "preclinical" Alzheimer's disease. Ann Neurol 1999 Mar;45(3):358-368.

(97) Yuan X, Russell T, Wood G, Desiderio DM. Analysis of the human lumbar cerebrospinal fluid proteome. Electrophoresis 2002;23(7-8):1185-1196.

(98) Sickmann A, Dormeyer W, Wortelkamp S, Woitalla D, Kuhn W, Meyer HE. Towards a high resolution separation of human cerebrospinal fluid. J Chromatogr B Analyt Technol Biomed Life Sci 2002 May 5;771(1-2):167-196.

(99) Davidsson P, Paulson L, Hesse C, Blennow K, Nilsson CL. Proteome studies of human cerebrospinal fluid and brain tissue using a preparative two-dimensional electrophoresis approach prior to mass spectrometry. Proteomics 2001 Mar;1(3):444-452.

(100) Davidsson P, Folkesson S, Christiansson M, et al. Identification of proteins in human cerebrospinal fluid using liquid-phase isoelectric focusing as a prefractionation step followed by two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionisation mass spectrometry. Rapid Commun Mass Spectrom 2002;16(22):2083-2088.

(101) Davidsson P, Sjogren M. Proteome studies of CSF in AD patients. Mech Ageing Dev 2006 Feb;127(2):133-137.

(102) Finehout EJ, Franck Z, Choe LH, Relkin N, Lee KH. Cerebrospinal fluid proteomic biomarkers for Alzheimer's disease. Ann Neurol 2007 Feb;61(2):120-129.

(103) Choe LH, Dutt MJ, Relkin N, Lee KH. Studies of potential cerebrospinal fluid molecular markers for Alzheimer's disease. Electrophoresis 2002 Jul;23(14):2247-2251.

(104) Castano EM, Roher AE, Esh CL, Kokjohn TA, Beach T. Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented

elderly subjects. Neurol Res 2006 Mar;28(2):155-163.

(105) Davidsson P, Westman-Brinkmalm A, Nilsson CL, et al. Proteome analysis of cerebrospinal fluid proteins in Alzheimer patients. Neuroreport 2002;13(5):611-615.

(106) Puchades M, Hansson SF, Nilsson CL, Andreasen N, Blennow K, Davidsson P. Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease. Brain Res Mol Brain Res 2003 Oct 21;118(1-2):140-146.

(107) Hye A, Lynham S, Thambisetty M, et al. Proteome-based plasma biomarkers for Alzheimer's disease. Brain 2006 Nov;129(Pt 11):3042-3050.

(108) Carrette O, Demalte I, Scherl A, et al. A panel of cerebrospinal fluid potential biomarkers for the diagnosis of Alzheimer's disease. Proteomics 2003 Aug;3(8):1486-1494.

(109) Ruetschi U, Zetterberg H, Podust VN, et al. Identification of CSF biomarkers for frontotemporal dementia using SELDI-TOF. Exp Neurol 2005 Dec;196(2):273-281.

(110) Simonsen AH, McGuire J, Podust VN, et al. Identification of a novel panel of cerebrospinal fluid biomarkers for Alzheimer's disease. Neurobiol Aging 2007 Feb 22;doi:10.1016/j.neurobiolaging.2007.01.011.

(111) Pasinetti GM, Ungar LH, Lange DJ, et al. Identification of potential CSF biomarkers in ALS. Neurology 2006 Apr 25;66(8):1218-1222.

(112) Ranganathan S, Williams E, Ganchev P, et al. Proteomic profiling of cerebrospinal fluid identifies biomarkers for amyotrophic lateral sclerosis. J Neurochem 2005 Dec;95(5):1461-1471.

(113) Huang JT, Leweke FM, Oxley D, et al. Disease biomarkers in cerebrospinal fluid of patients with first-onset psychosis. PLoS Med 2006 Nov;3(11):e428.

(114) Irani DN, Anderson C, Gundry R, et al. Cleavage of cystatin C in the cerebrospinal fluid of patients with multiple sclerosis. Ann Neurol 2006 Feb;59(2):237-247.

(115) Lewczuk P, Esselmann H, Groemer TW, et al. Amyloid beta peptides in cerebrospinal fluid as profiled with surface enhanced laser desorption/ionization time-of-flight mass spectrometry: evidence of novel biomarkers in Alzheimer's disease. Biol Psychiatry 2004 Mar 1;55(5):524-530.

(116) Maddalena AS, Papassotiropoulos A, Gonzalez-Agosti C, et al. Cerebrospinal fluid profile of amyloid beta peptides in patients with Alzheimer's disease determined by protein biochip technology. Neurodegener Dis 2004;1(4-5):231-235.

(117) Lewczuk P, Esselmann H, Meyer M, et al. The amyloid-beta (Abeta) peptide pattern in cerebrospinal fluid in Alzheimer's disease: evidence of a novel carboxyterminally elongated Abeta peptide. Rapid Commun Mass Spectrom 2003;17(12):1291-1296.

(118) Georganopoulou DG, Chang L, Nam JM, et al. Nanoparticle-based detection in cerebral spinal fluid of a soluble pathogenic biomarker for Alzheimer's disease. Proc Natl Acad

Sci U S A 2005 Feb 15;102(7):2273-2276.

(119) Wiltfang J, Esselmann H, Bibl M, et al. Highly conserved and disease-specific patterns of carboxyterminally truncated Abeta peptides 1-37/38/39 in addition to 1-40/42 in Alzheimer's disease and in patients with chronic neuroinflammation. J Neurochem 2002 May;81(3):481-496.

(120) Bibl M, Mollenhauer B, Esselmann H, et al. CSF amyloid-beta-peptides in Alzheimer's disease, dementia with Lewy bodies and Parkinson's disease dementia. Brain 2006 May;129(Pt 5):1177-1187.

(121) Portelius E, Zetterberg H, Andreasson U, et al. An Alzheimer's disease-specific beta-amyloid fragment signature in cerebrospinal fluid. Neurosci Lett 2006 Dec 6;409(3):215-219.

(122) Blennow K. Cerebrospinal fluid protein biomarkers for Alzheimer's disease. NeuroRx 2004 Apr;1(2):213-225.

(123) Teunissen CE, de Vente J, Steinbusch HW, de Bruijn C. Biochemical markers related to Alzheimer's dementia in serum and cerebrospinal fluid. Neurobiol Aging 2002 Jul;23(4):485-508.

(124) Frank RA, Galasko D, Hampel H, et al. Biological markers for therapeutic trials in Alzheimer's disease. Proceedings of the biological markers working group; NIA initiative on neuroimaging in Alzheimer's disease. Neurobiol Aging 2003 Jul;24(4):521-536.

(125) Pratico D, MY L, V, Trojanowski JQ, Rokach J, FitzGerald GA. Increased F2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. FASEB J 1998 Dec;12(15):1777-1783.

(126) Yao Y, Zhukareva V, Sung S, et al. Enhanced brain levels of 8,12-iso-iPF2alpha-VI differentiate AD from frontotemporal dementia. Neurology 2003 Aug 26;61(4):475-478.

(127) Montine TJ, Neely MD, Quinn JF, et al. Lipid peroxidation in aging brain and Alzheimer's disease. Free Radic Biol Med 2002 Sep 1;33(5):620-626.

(128) Pratico D, Clark CM, Lee VM, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-iso-iPF2alpha-VI in Alzheimer's disease: correlation of a noninvasive index of lipid peroxidation with disease severity. Ann Neurol 2000 Nov;48(5):809-812.

(129) Montine TJ, Kaye JA, Montine KS, McFarland L, Morrow JD, Quinn JF. Cerebrospinal fluid abeta42, tau, and f2-isoprostane concentrations in patients with Alzheimer disease, other dementias, and in age-matched controls. Arch Pathol Lab Med 2001 Apr;125(4):510-512.

(130) Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. Arch Neurol 2002 Jun;59(6):972-976.

(131) McGeer PL, McGeer EG. The inflammatory response system of brain: implications for ther-

apy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21(2):195-218.

(132) Jia JP, Meng R, Sun YX, Sun WJ, Ji XM, Jia LF. Cerebrospinal fluid tau, Abeta1-42 and inflammatory cytokines in patients with Alzheimer's disease and vascular dementia. Neurosci Lett 2005 Jul 22;383(1-2):12-16.

(133) Wada-Isoe K, Wakutani Y, Urakami K, Nakashima K. Elevated interleukin-6 levels in cerebrospinal fluid of vascular dementia patients. Acta Neurol Scand 2004 Aug;110(2):124-127.

(134) Rentzos M, Paraskevas GP, Kapaki E, et al. Interleukin-12 is reduced in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. J Neurol Sci 2006 Nov 15;249(2):110-114.

(135) Rentzos M, Zoga M, Paraskevas GP, et al. IL-15 is elevated in cerebrospinal fluid of patients with Alzheimer's disease and frontotemporal dementia. J Geriatr Psychiatry Neurol 2006 Jun;19(2):114-117.

(136) Grant P, Pant HC. Neurofilament protein synthesis and phosphorylation. J Neurocytol 2000 Nov;29(11-12):843-872.

(137) Hu YY, He SS, Wang XC, et al. Elevated levels of phosphorylated neurofilament proteins in cerebrospinal fluid of Alzheimer disease patients. Neurosci Lett 2002 Mar 8;320(3):156-160.

(138) Rosengren LE, Karlsson JE, Sjogren M, Blennow K, Wallin A. Neurofilament protein levels in CSF are increased in dementia. Neurology 1999 Mar 23;52(5):1090-1093.

(139) Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. J Neurochem 1996 Nov;67(5):2013-2018.

(140) Sjogren M, Blomberg M, Jonsson M, et al. Neurofilament protein in cerebrospinal fluid: a marker of white matter changes. J Neurosci Res 2001 Nov 1;66(3):510-516.

(141) Sjogren M, Rosengren L, Minthon L, Davidsson P, Blennow K, Wallin A. Cytoskeleton proteins in CSF distinguish frontotemporal dementia from AD. Neurology 2000 May 23;54(10):1960-1964.

(142) Sjogren M, Wallin A. Pathophysiological aspects of frontotemporal dementia--emphasis on cytoskeleton proteins and autoimmunity. Mech Ageing Dev 2001 Nov;122(16):1923-1935.

(143) De Jong D, Jansen RW, Pijnenburg YA, et al. CSF neurofilament proteins in the differential diagnosis of dementia. J Neurol Neurosurg Psychiatry 2007 Feb 21;10.1136/jnnp.2006.107326.

(144) Brettschneider J, Petzold A, Schottle D, Claus A, Riepe M, Tumani H. The neurofilament

heavy chain (NfH) in the cerebrospinal fluid diagnosis of Alzheimer's disease. Dement Geriatr Cogn Disord 2006;21(5-6):291-295.

(145) Pitschke M, Prior R, Haupt M, Riesner D. Detection of single amyloid beta-protein aggregates in the cerebrospinal fluid of Alzheimer's patients by fluorescence correlation spectroscopy. Nat Med 1998 Jul;4(7):832-834.

(146) Stenh C, Englund H, Lord A, et al. Amyloid-beta oligomers are inefficiently measured by enzyme-linked immunosorbent assay. Ann Neurol 2005 Jul;58(1):147-150.

(147) Santos AN, Torkler S, Nowak D, et al. Detection of amyloid-beta oligomers in human cerebrospinal fluid by flow cytometry and fluorescence resonance energy transfer. J Alzheimers Dis 2007 Mar;11(1):117-125.

(148) Holsinger RM, Lee JS, Boyd A, Masters CL, Collins SJ. CSF BACE1 activity is increased in CJD and Alzheimer disease versus [corrected] other dementias. Neurology 2006 Aug 22;67(4):710-712.

(149) Kehoe PG, Katzov H, Feuk L, et al. Haplotypes extending across ACE are associated with Alzheimer's disease. Hum Mol Genet 2003 Apr 15;12(8):859-867.

(150) Nielsen HM, Londos E, Minthon L, Janciauskiene SM. Soluble adhesion molecules and angiotensin-converting enzyme in dementia. Neurobiol Dis 2007 Apr;26(1):27-35.

(151) He M, Ohrui T, Maruyama M, et al. ACE activity in CSF of patients with mild cognitive impairment and Alzheimer disease. Neurology 2006 Oct 10;67(7):1309-1310.

(152) Mehta PD, Pirttila T, Patrick BA, Barshatzky M, Mehta SP. Amyloid beta protein 1-40 and 1-42 levels in matched cerebrospinal fluid and plasma from patients with Alzheimer disease. Neurosci Lett 2001 May 18;304(1-2):102-106.

(153) Freeman SH, Raju S, Hyman BT, Frosch MP, Irizarry MC. Plasma Abeta levels do not reflect brain Abeta levels. J Neuropathol Exp Neurol 2007 Apr;66(4):264-271.

(154) Mayeux R, Honig LS, Tang MX, et al. Plasma A[beta]40 and A[beta]42 and Alzheimer's disease: relation to age, mortality, and risk. Neurology 2003 Nov 11;61(9):1185-1190.

(155) Assini A, Cammarata S, Vitali A, et al. Plasma levels of amyloid beta-protein 42 are increased in women with mild cognitive impairment. Neurology 2004 Sep 14;63(5):828-831.

(156) Blasko I, Jellinger K, Kemmler G, et al. Conversion from cognitive health to mild cognitive impairment and Alzheimer's disease: Prediction by plasma amyloid beta 42, medial temporal lobe atrophy and homocysteine. Neurobiol Aging 2006 Oct 19;10.1016/j.neurobiolaging.2006.09.002.

(157) Fukumoto H, Tennis M, Locascio JJ, Hyman BT, Growdon JH, Irizarry MC. Age but not diagnosis is the main predictor of plasma amyloid beta-protein levels. Arch Neurol 2003 Jul;60(7):958-964.

(158) Oijen M.van, Hofman A, Soares HD, Koudstaal PJ, Breteler MM. Plasma Abeta(1-40) and Abeta(1-42) and the risk of dementia: a prospective case-cohort study. Lancet Neurol 2006 Aug;5(8):655-660.

(159) Graff-Radford NR, Crook JE, Lucas J, et al. Association of low plasma Abeta42/Abeta40 ratios with increased imminent risk for mild cognitive impairment and Alzheimer disease. Arch Neurol 2007 Mar;64(3):354-362.

(160) Blasko I, Kemmler G, Krampla W, et al. Plasma amyloid beta protein 42 in non-demented persons aged 75 years: effects of concomitant medication and medial temporal lobe atrophy. Neurobiol Aging 2005 Aug;26(8):1135-1143.

(161) Gluud C, Gluud LL. Evidence based diagnostics. BMJ 2005 Mar 26;330(7493):724-726.

(162) Bossuyt PM, Irwig L, Craig J, Glasziou P. Comparative accuracy: assessing new tests against existing diagnostic pathways. BMJ 2006 May 6;332(7549):1089-1092.

Chapter 4

Summary and general discussion

Chapter 4.1

Summary and general discussion

Summary

Alzheimer's disease and anti-inflammatory drugs

The objective of this part of the thesis was to explore the role of inflammation in the pathogenesis of Alzheimer's disease (AD), and to investigate whether anti-inflammatory drugs, especially nonsteroidal anti-inflammatory drugs (NSAIDs), are able to retard the progression of AD.

In **Chapter 2.1** we reviewed the different lines of research regarding AD and inflammation. In 1982, the first indications that inflammation plays a role in AD were found in post-mortem brains of AD patients. Additional neuropathological research revealed that various inflammatory mediators are found within and surrounding amyloid plaques in AD brains. Epidemiological studies consistently demonstrated that the use of NSAIDs prior to incipient disease is associated with a reduced risk for the development of AD. Research concerning the mechanism of action of NSAIDs in AD revealed that the effect of NSAIDs in AD is probably mediated by activation of the peroxisome proliferator-activated receptor-γ. In animal model studies it was demonstrated that the administration of NSAIDs to AD transgenic mice suppressed the formation of amyloid plaques and inflammatory mediators. In cultured cells, a subset of NSAIDs was found to lower the amount of amyloid β_{42} secretion. Together, these findings raised the suggestion that NSAIDs will be able not only to postpone AD onset (primary prevention) but also to retard AD progression. In 2000, when our randomized controlled trial with the NSAID indomethacin started (see chapter 2.2), only one small clinical trial had shown that treatment with NSAIDs significantly delayed cognitive decline in AD patients. We concluded that large randomized double-blind placebo-controlled trials were needed to demonstrate a definite beneficial effect of NSAIDs in AD.

In **chapter 2.2** we described the methods and results of a double-blind, randomized, placebo-controlled trial with indomethacin in AD patients. Our objective was to assess whether treatment with the NSAID indomethacin slows cognitive decline in patients with AD. The study was conducted between May 2000 and September 2005 in two hospitals in the Netherlands. Fifty-one patients with mild to moderate AD were enrolled into the study. Patients received 100 mg indomethacin or placebo daily for 12 months. Additionally, all patients received omeprazole as a

gastroprotectant. The primary outcome measure was the change from baseline on the cognitive subscale of the AD Assessment Scale (ADAS-cog) over the one year treatment period. Secondary outcome measures included the Mini-Mental State Examination, the Clinician's Interview Based Impression of Change with caregiver input, the noncognitive subscale of the ADAS, the Neuropsychiatric Inventory, and the Interview for Deterioration in Daily life in Dementia. Unfortunately, considerable recruitment problems of participants were encountered, leading to an underpowered study. In the placebo group, 19 out of 25 patients completed the study, and in the indomethacin group 19 out of 26. The deterioration on the ADAS-cog was less in the indomethacin group (7.8 ± 7.6), than in the placebo group (9.3 ± 10.0). This difference (1.5 points; CI –4.5 - 7.5) was not statistically significant; neither were any of the secondary outcome measures. Indomethacin, in combination with omeprazole, was reasonably well tolerated in the elderly trial patients without serious gastrointestinal tract events. We concluded that the results of our study were inconclusive with respect to the hypothesis that indomethacin slows the progression of AD.

In **Chapter 2.3** we investigated the external validity of the results of our previously conducted randomized controlled trial (RCT), and we evaluated the generalizability of the results of other drug-trials in AD patients. All AD patients that participated in our RCT with indomethacin (RCT group, n = 51), were compared with all remaining AD patients seen at our memory clinic for diagnosis and treatment during the four-year recruitment period of the trial (control group, n = 128). Characteristics of these patients, such as medication use, comorbidity, results of physical and neurological examination, were collected. The Cumulative Illness Rating Scale for Geriatrics was used to assess the presence and severity of comorbidity. Furthermore, 72 RCTs with AD patients in which various drugs were tested, were selected from the literature for further comparisons. We found that the age of the patients in the RCT group was significantly lower (72.4 ± 8.0 years) than in the control group (76.1 ± 6.5 years; $p<0.01$). Furthermore, patients in the RCT group had fewer disabilities and comorbid conditions, and were taking less medication, than those in the control group. In 62 out of 72 evaluated other RCTs mean age of participating patients was < 76 years. In only 11 RCT articles some information was available on medication use or comorbidity of participants. Thus, we concluded that the external validity of the results of our RCT with indomethacin, and of many other RCTs with

AD patients, is limited. Also, care should be taken to extrapolate conclusions from clinical trials to a general population of AD patients.

Cerebrospinal fluid diagnosis in Alzheimer's disease

Differentiating AD from other dementia disorders, such as vascular dementia (VaD), frontotemporal lobar degeneration (FTLD), and dementia with Lewy bodies (DLB) is becoming increasingly important. An accurate and early diagnosis is essential for appropriate support and treatment of dementia patients, since symptomatic drugs are already available for AD patients, and neuroprotective drugs are being developed. However, the clinical diagnostic criteria currently used for the differentiation between AD and other dementias have disappointing sensitivity and specificity.

The objective of this part of the thesis was to investigate whether the analysis of biomarkers, especially in cerebrospinal fluid (CSF), could be helpful in discriminating AD from other dementias.

In **chapter 3.1** we focused on the differentiation of AD from VaD. The objective of this study was to investigate whether CSF levels of total tau protein (t-tau), amyloid β_{42} protein ($A\beta_{42}$) and tau phosphorylated at threonine 181 (p-tau_{181}) are useful biomarkers to distinguish AD patients from VaD patients. We measured CSF levels of p-tau_{181}, $A\beta_{42}$, and t-tau in patients with mild to moderate AD (n = 61) and VaD (n = 25), and 30 control subjects (NC). Optimal differentiation between AD and VaD was achieved by using the ratio of the CSF levels of $A\beta_{42}$ and p-tau_{181} (Q $A\beta_{42}$/p-tau) with sensitivity, specificity, positive and negative predictive values all ≥ 85%. We concluded that our results support further efforts to prospectively validate the use of Q $A\beta_{42}$/p-tau as a biomarker to differentiate between AD and VaD.

In **chapter 3.2** we investigated the diagnostic value of CSF neurofilament (NF) protein analysis in relatively young dementia patients to differentiate between FTLD and early-onset AD (EAD; onset ≤ 65 years of age), and in elderly dementia patients between DLB and late-onset AD (LAD; onset > 65 years of age). NF proteins are major cytoskeletal constituents of neurons. Increased CSF NF levels may reflect neuronal damage during degeneration. In CSF of 28 FTLD, 37 EAD, 18 DLB, and 33 LAD patients, and 26 control subjects, we analysed NF light chain (NFL), phosphorylated NF heavy chain (pNFH), $A\beta_{42}$, t-tau, and p-tau_{181}. CSF NFL levels were higher in FTLD patients compared to EAD patients ($p < 0.001$), and diagnostic accuracy of p-tau_{181}

and $A\beta_{42}$ analysis improved with the addition of NFL analysis (sensitivity 86%, specificity 100%). CSF pNFH levels were elevated in DLB, LAD, and FTLD compared to controls ($p < 0.05$), but no significant differences were found between the various dementia groups. We concluded that in the diagnostic workup of relatively young dementia patients, CSF NFL levels may play a role in the discrimination between FTLD and EAD, especially in combination with $A\beta_{42}$ and p-tau_{181} analysis.

Chapter 3.3 offers a comprehensive review of the literature on neurochemical biomarkers for AD. We summarized the current state-of-the-art of these biomarkers. Predominantly, these biomarkers are cerebrospinal fluid biomarkers directly related to the pathofysiology of this disorder (such as amyloid β protein, tau protein). We particularly paid attention to the innovations in this area that have been made in technological aspects during the past 5 years (e.g. multiplex analysis of biomarkers, proteomics), to the discovery of novel, potential biomarkers (e.g amyloid β oligomers, isoprostanes), and to the extension of this research towards identification of biomarkers in plasma.

General discussion

Alzheimer's disease and anti-inflammatory drugs

AD is a complex and devastating neurodegenerative disorder for which the currently available drugs offer only a modest symptomatic effect. Animal studies, in vitro data, and epidemiologic evidence strongly supports the hypothesis that NSAIDs have disease-modifying properties (**chapter 2.1**). However, our randomized clinical trial with the NSAID indomethacin failed to show an effect on the progression of AD (**chapter 2.2**). Although our trial was hampered by extensive exclusion criteria which resulted in a underpowered study, low external validity (**chapter 2.3**), and inconclusive results, the disappointing results of other trials with various NSAIDs (diclofenac/misoprostol, ibuprofen, naproxen, rofecoxib, nimesulide, and celecoxib) sheds serious doubts on the hypothesis.(1-6) Nevertheless, there is still a vast belief among many researchers that NSAIDs can prevent or slow the progression of AD.

The failure of clinical trials with NSAIDs in AD patients has led to many speculations why there was no response. Several possible reasons have been suggested. First, the dosage of the drug used, or the level that the drug of interest reaches in the central nervous system could be too low. Especially naproxen was given at a low-dose to prevent side-effects, which might be responsible for the failure of this trial. (2) A second reason could be the timing of the intervention. It may be questioned whether anti-inflammatory treatment will be efficacious in treating symptomatic AD. Even when given in an earlier stage of the disease, there seems not to be an effect; In a randomized controlled study in patients with mild cognitive impairment (MCI), rofecoxib could not delay a diagnosis of AD in patients with MCI.(7) Possibly NSAIDs only have primary preventive effects, and are no longer effective in patients with developing or established disease. However, the results of the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT), in which men and women aged 70 years and older with a family history of AD were randomly assigned to receive naproxen, celecoxib or placebo, did not support a preventive effect either.(8) These results appear to be inconsistent with the epidemiological findings that originally provided the rationale for this trial (**chapter 2.1**). In that case, the final option is that NSAIDs might exert protective effects only if given numerous years before the time when symptoms would otherwise develop. This agrees with the results of the Rotter-

dam and Cache County observational studies, which showed protective effects with more distant, but not recent, use of NSAIDs.(9;10) A recent epidemiological study in an older cohort (median age 74.8 years) found an increased incidence of dementia and AD in heavy NSAID users,(11) which might also be an argument for the use of NSAIDs a long time before AD symptoms develop.

A different explanation for the negative results of the ADAPT study and other trials with NSAIDs concerns the pharmacological and pharmacokinetic properties of the drugs being used. Initially, investigators were mainly interested in the cyclooxygenase (COX)-inhibiting properties of NSAIDs (**chapter 2.1**). Selective COX-2 inhibitors with a more favorable side-effects profile were being developed. Clinical trials followed with celecoxib and rofecoxib in AD patients, patients with MCI, and elderly patients with a family history of AD, but none showed a significant effect.(2;4;6;7;12) As COX-inhibition by NSAIDs appeared not to play an important role, attention shifted towards an alternative mechanism of action of NSAIDs: the activation of the peroxisome proliferator-activated receptor-gamma (PPAR-γ), a nuclear receptor (**chapter 2.1**). Most classical NSAIDs, such as indomethacin, naproxen, and ibuprofen are PPAR-γ agonists, with the exception of diclofenac, which is only a partial agonist. (13;14) However, all clinical trials using NSAIDs that activate PPAR-γ failed to show an effect (**chapter 2.2**).(2;3;8;12)

A subset of NSAIDs, such as ibuprofen or flurbiprofen, have $A\beta_{42}$-lowering properties by modulating the activity of gamma-secretase (**chapter 2.1**). Although ibuprofen failed to show an effect in AD patients, evidence was found of a dose-related effect in patients with mild AD in a phase II trial with tarenflurbil (R-flurbiprofen). (3;15) However, the results of the largest and longest phase III trial in mild AD patients with tarenflurbil were very disappointing; Tarenflurbil failed to distinguish itself from placebo in its primary endpoints of slowing decline in AD.(16) A simple explanation of this failure might be that the gamma-secretase is not the right target for therapy or that, in general, blocking $A\beta_{42}$ does not produce clinical benefits in AD.

Conclusion and future perspectives

Since many clinical trials testing various NSAIDs at different stages of AD (pre-symptomatic, MCI, mild and moderate AD) failed to show an effect, there is not much hope left that a NSAID can either treat or prevent AD. Therefore, we do not recom-

mend further treatment trials with NSAIDs in AD patients. Nevertheless, a primary prevention trial with ibuprofen in middle aged individuals (approximately between the ages of 40 and 60 years) is still warranted to further investigate the effect of long-term NSAID use on risk of AD.

Cerebrospinal fluid diagnosis in Alzheimer's disease

Despite extensive research on this topic, the most successful neurochemical biomarkers for AD in CSF still remain $A\beta_{42}$, t-tau, and p-tau_{181} **(chapter 3.3)**. Test characteristics are excellent to differentiate AD from controls using these CSF biomarkers. Moreover, there is increasing evidence that levels of these biomarkers correlate with clinical and neuropathological features in AD patients. In individuals with very mild AD, lower CSF $A\beta_{42}$ levels, high t-tau or $ptau_{181}$ levels, or high ratio of CSF levels of t-tau and $A\beta_{42}$ quantitatively predict more rapid progression of cognitive deficits and dementia.(17) Pathological CSF levels of $A\beta_{42}$, t-tau, and $ptau_{181}$ are strongly associated with future development of AD in patients with MCI.(18-20) Also, CSF $A\beta_{42}$ levels correlate inversely with total Aβ load in the brain, and CSF t-tau levels correlate with the presence of neocortical neurofibrillary tangles.(21) Furthermore, increased CSF p-tau levels are independently associated with faster subsequent disease progression, as reflected by higher hippocampal atrophy rates.(22) A subgroup of patients with extremely high CSF t-tau and $ptau_{181}$ levels shows a distinct cognitive profile with more severe impairment of memory, mental speed, and executive functions, which cannot be explained by disease severity.(23) Altogether, CSF $A\beta_{42}$, t-tau, and p-tau_{181} have proven their value in AD in many ways.

When using these biomarkers in clinical practice, however, standardized (pre) analytical procedures should be followed for correct and reliable analysis of $A\beta_{42}$, t-tau, and p-tau_{181} levels. CSF withdrawal should be performed in polypropylene tubes, and at a standardized time point of the day, because of diurnal fluctuations of Aβ concentrations.(24;25) CSF samples must be centrifuged, aliquoted and stored at –80°C as soon as possible after withdrawal.(26) Furthermore, the same assay should always be used to decrease intracenter, and intercenter variation.(27)

Differentiating AD from other dementia disorders, including DLB, FTLD and VaD, using CSF $A\beta_{42}$, t-tau, and p-tau_{181} is more difficult, although the ratio of CSF

levels of $A\beta_{42}$ and p-tau_{181} (Q $A\beta_{42}$/p-tau) can be helpful for the differentiation between AD and VaD with sensitivity, specificity, positive and negative predictive values all ≥ 85% (**chapter 3.1**). In some cases, the t-tau/p-tau_{181} ratio in CSF may contribute to the clinical distinction between DLB and AD, but the value of the markers is still limited.(28) Thus, additional biomarkers are needed. In the diagnostic workup of relatively young dementia patients, we found that CSF NFL levels in combination with $A\beta_{42}$ and p-tau_{181} analysis are helpful in the discrimination between FTLD and EAD (**chapter 3.2**). However, since our review was published in 2007 (**chapter 3.3**), no promising novel biomarkers have been introduced. Now, attention has shifted towards combining neurochemical biomarkers in CSF, structural neuroimaging with MRI, and molecular neuroimaging with PET in order to optimize the diagnosis of AD, and other dementia syndromes. Already, revised research criteria for AD have been proposed that implements these biomarkers.(29)

Conclusion and future perspectives

The value of the CSF biomarkers $A\beta_{42}$, t-tau, and p-tau_{181} in AD is well established. However, the differentiation between AD, DLB, FTLD and VaD using CSF biomarkers still needs to be improved. Further research should focus on finding specific CSF biomarkers in FTLD, VaD, and DLB. Preferably, these biomarkers should correlate with neuropathological findings in these patients. Novel technologies, including mass spectrometry analysis, may lead to the discovery of new biomarkers. Probably the most promising type of new biomarkers are still to be found in CSF.

References

(1) Scharf S, Mander A, Ugoni A, Vajda F, Christophidis N. A double-blind, placebo-controlled trial of diclofenac/misoprostol in Alzheimer's disease. Neurology 1999 Jul 13;53(1):197-201.

(2) Aisen PS, Schafer KA, Grundman M, et al. Effects of rofecoxib or naproxen vs placebo on Alzheimer disease progression: a randomized controlled trial. JAMA 2003 Jun 4;289(21):2819-2826.

(3) Pasqualetti P, Bonomini C, Dal FG, et al. A randomized controlled study on effects of ibuprofen on cognitive progression of Alzheimer's disease. Aging Clin Exp Res 2009 Apr;21(2):102-110.

(4) Reines SA, Block GA, Morris JC, et al. Rofecoxib: no effect on Alzheimer's disease in a 1-year, randomized, blinded, controlled study. Neurology 2004 Jan 13;62(1):66-71.

(5) Aisen PS, Schmeidler J, Pasinetti GM. Randomized pilot study of nimesulide treatment in Alzheimer's disease. Neurology 2002 Apr 9;58(7):1050-1054.

(6) Soininen H, West C, Robbins J, Niculescu L. Long-term efficacy and safety of celecoxib in Alzheimer's disease. Dement Geriatr Cogn Disord 2007;23(1):8-21.

(7) Thal LJ, Ferris SH, Kirby L, et al. A randomized, double-blind, study of rofecoxib in patients with mild cognitive impairment. Neuropsychopharmacology 2005 Jun;30(6):1204-1215.

(8) Martin BK, Szekely C, Brandt J, et al. Cognitive function over time in the Alzheimer's Disease Anti-inflammatory Prevention Trial (ADAPT): results of a randomized, controlled trial of naproxen and celecoxib. Arch Neurol 2008 Jul;65(7):896-905.

(9) in 't Veld, Launer LJ, Hoes AW, et al. NSAIDs and incident Alzheimer's disease. The Rotterdam Study. Neurobiol Aging 1998 Nov;19(6):607-611.

(10) Anthony JC, Breitner JC, Zandi PP, et al. Reduced prevalence of AD in users of NSAIDs and H2 receptor antagonists: the Cache County study. Neurology 2000 Jun 13;54(11):2066-2071.

(11) Breitner JC, Haneuse SJ, Walker R, et al. Risk of dementia and AD with prior exposure to NSAIDs in an elderly community-based cohort. Neurology 2009 Jun 2;72(22):1899-1905.

(12) ADAPT Research Group, Lyketsos CG, Breitner JC, et al. Naproxen and celecoxib do not prevent AD in early results from a randomized controlled trial. Neurology 2007 May 22;68(21):1800-1808.

(13) Jaradat MS, Wongsud B, Phornchirasilp S, et al. Activation of peroxisome proliferator-activated receptor isoforms and inhibition of prostaglandin H(2) synthases by ibuprofen, naproxen, and indomethacin. Biochem Pharmacol 2001 Dec 15;62(12):1587-1595.

(14) Adamson DJ, Frew D, Tatoud R, Wolf CR, Palmer CN. Diclofenac antagonizes peroxisome

proliferator-activated receptor-gamma signaling. Mol Pharmacol 2002 Jan;61(1):7-12.

(15) Wilcock GK, Black SE, Hendrix SB, Zavitz KH, Swabb EA, Laughlin MA. Efficacy and safety of tarenflurbil in mild to moderate Alzheimer's disease: a randomised phase II trial. Lancet Neurol 2008 Jun;7(6):483-493.

(16) Wilcock GK, Black SE, Balch AH, et al. Safety and efficacy of tarenflurbil in subjects with mild Alzheimer's disease: Results from an 18-month multi-center phase 3 trial. Alzheimers Dement 2009 Jul;5(4):P86Abs.

(17) Snider BJ, Fagan AM, Roe C, et al. Cerebrospinal fluid biomarkers and rate of cognitive decline in very mild dementia of the Alzheimer type. Arch Neurol 2009 May;66(5):638-645.

(18) Hansson O, Zetterberg H, Buchhave P, Londos E, Blennow K, Minthon L. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: a follow-up study. Lancet Neurol 2006 Mar;5(3):228-234.

(19) Visser PJ, Verhey F, Knol DL, et al. Prevalence and prognostic value of CSF markers of Alzheimer's disease pathology in patients with subjective cognitive impairment or mild cognitive impairment in the DESCRIPA study: a prospective cohort study. Lancet Neurol 2009 Jul;8(7):619-627.

(20) Mattsson N, Zetterberg H, Hansson O, et al. CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. JAMA 2009 Jul 22;302(4):385-393.

(21) Tapiola T, Alafuzoff I, Herukka SK, et al. Cerebrospinal fluid {beta}-amyloid 42 and tau proteins as biomarkers of Alzheimer-type pathologic changes in the brain. Arch Neurol 2009 Mar;66(3):382-389.

(22) Henneman WJ, Vrenken H, Barnes J, et al. Baseline CSF p-tau levels independently predict progression of hippocampal atrophy in Alzheimer disease. Neurology 2009 Sep 22;73(12):935-940.

(23) Vlies AEvd, Verwey NA, Bouwman FH, et al. CSF biomarkers in relationship to cognitive profiles in Alzheimer disease. Neurology 2009 Mar 24;72(12):1056-1061.

(24) Vanderstichele H, Blennow K, D'Heuvaert N, et al. Development of a specific diagnostic test for the measurement of beta-amyloid(1-42) in CSF. In: Fisher A, Hanin I, Yoshida M, eds. Progress in Alzheimer's and Parkinson's diseases. New York: Plenum Publishing Group, 1998:773-778.

(25) Bateman RJ, Wen G, Morris JC, Holtzman DM. Fluctuations of CSF amyloid-beta levels: implications for a diagnostic and therapeutic biomarker. Neurology 2007 Feb 27;68(9):666-669.

(26) Schoonenboom NS, Mulder C, Vanderstichele H, et al. Effects of processing and storage conditions on amyloid beta (1-42) and tau concentrations in cerebrospinal fluid: implica-

tions for use in clinical practice. Clin Chem 2005 Jan;51(1):189-195.

(27) Verwey NA, van der Flier WM, Blennow K, et al. A worldwide multicentre comparison of assays for cerebrospinal fluid biomarkers in Alzheimer's disease. Ann Clin Biochem 2009 May;46(Pt 3):235-240.

(28) Mollenhauer B, Bibl M, Wiltfang J, et al. Total tau protein, phosphorylated tau (181p) protein, beta-amyloid(1-42), and beta-amyloid(1-40) in cerebrospinal fluid of patients with dementia with Lewy bodies. Clin Chem Lab Med 2006;44(2):192-195.

(29) Dubois B, Feldman HH, Jacova C, et al. Research criteria for the diagnosis of Alzheimer's disease: revising the NINCDS-ADRDA criteria. Lancet Neurol 2007 Aug;6(8):734-746.

Chapter 4.2

Samenvatting

Samenvatting

De ziekte van Alzheimer en anti-infammatoire geneesmiddelen

Het doel van het eerste deel van het proefschrift was het bestuderen van de aspecten en de rol van ontstekingsreacties in de hersenen bij mensen met de ziekte van Alzheimer, en vervolgens te onderzoeken of ontstekingsremmende geneesmiddelen, met name niet-steroide anti-inflammatoire middelen (NSAID's), de progressie van deze ziekte kunnen vertragen.

In **hoofdstuk 2.1** hebben we de verschillende onderzoekslijnen betreffende de ziekte van Alzheimer en ontsteking samengevat. In 1982 werden de eerste aanwijzingen gevonden dat ontstekingsprocessen in de hersenen een rol spelen bij de ziekte van Alzheimer. Daarop volgend neuropathologisch onderzoek toonde aan dat diverse ontstekingsmediatoren aanwezig zijn in en rondom amyloïde plaques in hersenen van patiënten met de ziekte van Alzheimer. Diverse epidemiologische studies toonden aan dat het gebruik van NSAID's voorafgaand aan het begin van de ziekte geassocieerd is met een verminderd risico op het ontwikkelen van de ziekte van Alzheimer. Bij onderzoek naar het werkingsmechanisme van NSAID's bij de ziekte van Alzheimer werd gevonden dat het effect van NSAID's bij deze ziekte waarschijnlijk gemedieerd wordt door de activatie van de peroxisoom-proliferator-geactiveerde receptor-γ. Toepassing van NSAID's in een muizenmodel van de ziekte van Alzheimer bleek de vorming van amyloïde plaques en ontstekingsmediatoren te remmen. In gekweekte cellen bleek tevens dat diverse NSAID's de hoeveelheid amyloïd β_{42} afgifte kunnen remmen. Bovenstaande bevindingen deden vermoeden dat NSAID's in staat zijn om niet alleen het ontstaan van de ziekte van Alzheimer uit te stellen (primaire preventie), maar ook de progressie van deze ziekte te vertragen. Tijdens de start van onze dubbelblinde gerandomiseerde placebogecontroleerde klinische trial met de NSAID indometacine in het jaar 2000 (zie hoofdstuk 2.2) was nog maar bij 1 kleine klinische studie bij alzheimerpatiënten een significante afname van de cognitieve achteruitgang gevonden bij gebruik van een NSAID. Wij concludeerden dat grote gerandomiseerde dubbelblinde placebogecontroleerde klinische trials nodig waren om een zeker positief effect van het gebruik van NSAID's bij de ziekte van Alzheimer aan te tonen.

In **hoofdstuk 2.2** beschrijven we de methoden en resultaten van zo'n dubbelblinde gerandomiseerde placebogecontroleerde klinische trial met indometacine in alzheimerpatiënten. Het doel van de studie was te onderzoeken of behandeling met de NSAID indometacine de cognitieve achteruitgang zou vertragen bij patiënten met de ziekte van Alzheimer. Deze studie werd uitgevoerd in twee ziekenhuizen in Nederland in de periode van mei 2000 tot september 2005. Eenenvijftig patiënten met lichte tot matig ernstige ziekte van Alzheimer werden geïncludeerd in de studie. Patiënten werden behandeld met indometacine (100 mg per dag) of placebo. Daarnaast kregen alle patiënten de maagbeschermer omeprazol voorgeschreven. De primaire uitkomstmaat voor de studie was de verandering na 1 jaar ten opzichte van de uitgangssituatie, gemeten met de cognitieve deelschaal van de "Alzheimer's Disease Assessment Scale" (ADAS-cog). De "Mini-Mental State Examination", de "Clinician's Interview Based Impression of Change with caregiver input", de niet-cognitieve deelschaal van de ADAS, de "Neuropsychiatric Inventory", en de "Interview for Deterioration in Daily life in Dementia" werden gebruikt als secundaire uitkomstmaat. Helaas werden aanzienlijke problemen ondervonden met het includeren van patiënten, waardoor de studie onvoldoende statistische power had. In de placebo groep hebben 19 van de 25 patiënten de studie afgerond en in de indometacine groep 19 van de 26 patiënten. De achteruitgang gemeten met de ADAS-cog was minder in de indometacine groep (7.8 ± 7.6) dan in de placebo groep (9.3 ± 10.0). Dit verschil (1.5 punten; CI –4.5 - 7.5) was echter niet statistisch significant; evenmin als de secundaire uitkomstmaten. Indometacine, in combinatie met omeprazol, werd vrij goed verdragen in de oudere deelnemende patiënten, zonder ernstige gastro-intestinale bijwerkingen. Wij moesten concluderen dat de resultaten van onze studie geen uitspraak toelieten aangaande de hypothese dat indometacine de progressie van de ziekte van Alzheimer zou vertragen.

In **hoofdstuk 2.3** onderzochten we de externe validiteit van de resultaten van onze tevoren verrichtte dubbelblinde gerandomiseerde placebogecontroleerde klinische trial en evalueerden we de generaliseerbaarheid van de resultaten van andere klinische trials bij alzheimerpatiënten. Alle alzheimerpatiënten die deelnamen aan onze klinische trial met indometacine (trial groep, n = 51) werden vergeleken met alle overgebleven alzheimerpatiënten die op de geheugenpolikliniek waren

gezien gedurende de vier jaar durende inclusie periode van de trial (controle groep, n = 128). De karakteristieken van deze patiënten, zoals medicatie gebruik, comorbiditeit, resultaten van algemeen lichamelijk en neurologisch onderzoek, werden verzameld. De "Cumulative Illness Rating Scale for Geriatrics" (CIRS-G) werd gebruikt om de aanwezigheid en de ernst van de comobiditeit te bepalen. Ter vergelijking werden verder nog 72 gerandomiseerde placebogecontroleerde klinische trials uit de literatuur geselecteerd waarin verschillende geneesmiddelen zijn getest bij alzheimerpatiënten. De leeftijd van de patiënten in de trial groep (72.4 ± 8.0 jaar) bleek significant lager te zijn dan in de controle groep (76.1 ± 6.5 jaar; $p<0.01$). Bovendien bleken patiënten in de trial groep minder handicaps en comorbide aandoeningen te hebben en minder geneesmiddelen te gebruiken dan de patiënten in de controle groep. In 62 van de 72 geëvalueerde overige klinische trials was de gemiddelde leeftijd van de deelnemende patiënten < 76 jaar. Uiteindelijk hebben wij geconcludeerd dat de externe validiteit van de resultaten van onze klinische trial met indometacine, evenals van vele andere klinische trials, beperkt is. Bovendien is voorzichtigheid geboden met het extrapoleren van conclusies van klinische trials naar de doorsnee populatie alzheimerpatiënten.

Liquor cerebrospinalis diagnose bij de ziekte van Alzheimer

Het onderscheiden van de ziekte van Alzheimer van andere dementie syndromen, zoals vasculaire dementie, frontotemporale lobaire degeneratie en dementie met Lewy lichaampjes, wordt steeds belangrijker. Een accurate en vroege diagnose is essentieel voor de juiste ondersteuning en behandeling van dementie patiënten, aangezien er al symptomatische geneesmiddelen beschikbaar zijn voor alzheimerpatiënten en neuroprotectieve geneesmiddelen in ontwikkeling zijn. Echter, de klinische criteria die momenteel gebruikt worden om de ziekte van Alzheimer te differentiëren van andere dementie syndromen hebben een teleurstellende sensitiviteit en specificiteit.

Het doel van dit deel van het proefschrift was te onderzoeken of de analyse van biomarkers, met name in de liquor cerebrospinalis (hersenvocht), behulpzaam zou kunnen zijn bij het onderscheiden van de ziekte van Alzheimer van andere dementie syndromen.

In hoofdstuk 3.1 hebben we ons gericht op de differentiatie tussen de ziekte van Alzheimer en vasculaire dementie. Het doel van deze studie was te onderzoeken of de concentraties van het totale tau eiwit (t-tau), het amyloïd β_{42} eiwit ($A\beta_{42}$) en gefosforyleerd tau op threonine 181 (p-tau_{181}) waardevolle biomarkers zijn waarmee alzheimerpatiënten van patiënten met vasculaire dementie onderscheiden kunnen worden. We maten de liquor concentraties van p-tau_{181}, $A\beta_{42}$, en t-tau bij patiënten met milde tot matig ernstige ziekte van Alzheimer (n = 61), bij patiënten met vasculaire dementie (n = 25) en bij controle personen (n = 30). De meest optimale differentiatie tussen de ziekte van Alzheimer en vasculaire dementie werd bereikt met behulp van de ratio van de liquor concentraties van $A\beta_{42}$ en p-tau_{181} (Q $A\beta_{42}$/p-tau), met een sensitiviteit, specificiteit, positief en negatief voorspellende waarde van ≥ 85%. Wij concludeerden dat onze resultaten verdere pogingen ondersteunen om prospectief het gebruik van Q $A\beta_{42}$/p-tau, als biomarker om te differentiëren tussen de ziekte van Alzheimer en vasculaire dementie, te valideren.

In **hoofdstuk 3.2** hebben we de diagnostische waarde onderzocht van neurofilament (NF) eiwit analyse in de liquor van relatief jonge dementie patiënten om te kunnen differentiëren tussen frontotemporale lobaire degeneratie (FTLD) en de ziekte van Alzheimer die op jonge leeftijd is ontstaan (≤ 65 jaar; EAD). Onder oudere dementie patiënten is gekeken naar de differentiatie tussen dementie met Lewy lichaampjes (DLB) en de ziekte van Alzheimer die op latere leeftijd is ontstaan (> 65 jaar; LAD). NF eiwitten zijn belangrijke cytoskelet bestanddelen van neuronen. Verhoogde NF concentraties in de liquor duiden op neuronale schade tijdens degeneratie. In de liquor van 28 FTLD, 37 EAD, 18 DLB, en 33 LAD patiënten, alsmede 26 controle personen, analyseerden we lichte keten NF (NFL), gefosforyleerd zware keten NF (pNFH), $A\beta_{42}$, t-tau en p-tau_{181}. NFL concentraties in de liquor bleken hoger in FTLD patiënten vergeleken met EAD patiënten ($p < 0.001$), en de diagnostische accuratesse van de p-tau_{181} en $A\beta_{42}$ analyse verbeterde met de toevoeging van de NFL analyse (sensitiviteit 86%, specificiteit 100%). pNFH concentraties in de liquor waren verhoogd in DLB, LAD en FTLD vergeleken met controles ($p < 0.05$), maar er werden geen significante verschillen gevonden tussen de verschillende dementie groepen onderling. Wij concludeerden dat in de diagnostische workup van relatief jonge dementie patiënten de analyse van NF concentraties in de liquor een rol kan

spelen in het onderscheid tussen FTLD en EAD, met name in combinatie met $A\beta_{42}$ en p-tau_{181} analyse.

Hoofdstuk 3.3 betreft een uitgebreid overzicht van de literatuur op het gebied van neurochemische biomarkers voor de ziekte van Alzheimer. De laatste ontwikkelingen op het gebied van deze biomarkers zijn samengevat. Hoofdzakelijk betreft het biomarkers in de liquor cerebrospinalis, die direct gerelateerd zijn aan de pathofysiologie van deze ziekte (zoals amyloïd β en tau eiwit). We hebben in het bijzonder aandacht besteed aan de technische innovaties van de afgelopen jaren op dit gebied (zoals multiplex analyse van biomarkers, proteomics), aan de ontdekking van nieuwe potentiele biomarkers (zoals amyloïd β oligomeren, isoprostanen) en aan de uitbreiding van onderzoek richting de identificatie van biomarkers in het bloed.

Chapter 5

List of publications

List of publications

(1) Metman LV, Blanchet PJ, De Jong D, Mouradian MM, Chase TN. Effect of the putative dopamine D1 agonist and D2 antagonist FCE 23884 on Parkinson's disease. Mov Disord 1996 May;11(3):257-260.

(2) De Jong D, Jansen RW, Verbeek MM, Kremer HP. The possible suppression of Alzheimer's disease by nonsteroidal anti-inflammatory drugs. Ned Tijdschr Geneeskd 2002 Nov 2;146(44):2074-2078.

(3) Verbeek MM, De Jong D, Kremer HP. Brain-specific proteins in cerebrospinal fluid for the diagnosis of neurodegenerative diseases. Ann Clin Biochem 2003 Jan;40(Pt 1):25-40.

(4) Rosso SM, Donker KL, Baks T, et al. Frontotemporal dementia in The Netherlands: patient characteristics and prevalence estimates from a population-based study. Brain 2003 Sep;126(Pt 9):2016-2022.

(5) Abdo WF, De Jong D, Hendriks JC, et al. Cerebrospinal fluid analysis differentiates multiple system atrophy from Parkinson's disease. Mov Disord 2004 May;19(5):571-579.

(6) van Horssen J, De Jong D, de Waal RM, et al. Cerebral amyloid angiopathy with severe secondary vascular pathology: a histopathological study. Dement Geriatr Cogn Disord 2005;20(5):321-330.

(7) De Jong D, Jansen RWMM, Kremer HPH, Verbeek MM. Cerebrospinal fluid amyloid β42/phosphorylated tau ratio discriminates between Alzheimer's disease and vascular dementia. J Gerontol A Biol Sci Med Sci 2006;61(7):755-758.

(8) De Jong D, Jansen RW, Pijnenburg YA, et al. CSF neurofilament proteins in the differential diagnosis of dementia. J Neurol Neurosurg Psychiatry 2007 Feb 21;10.1136/jnnp.2006.107326.

(9) Reijn TS, Olde Rikkert M, Van Geel WJ, De Jong D, Verbeek MM. Diagnostic Accuracy of ELISA and xMAP Technology for Analysis of Amyloid {beta}42 and Tau Proteins. Clin Chem 2007 May;53(5):859-865.

(10) De Jong D, Kremer BP, Olde Rikkert MG, Verbeek MM. Current state and future directions of neurochemical biomarkers for Alzheimer's disease. Clin Chem Lab Med 2007;45(11):1421-1434.

(11) De Jong D, Jansen R, Hoefnagels W, et al. No effect of one-year treatment with indomethacin on Alzheimer's disease progression: a randomized controlled trial. PLoS ONE 2008;3(1):e1475.

(12) De Jong D, Kremer HPH, Verbeek MM, Borm GF, Jansen RWMM. External validity of a randomized controlled trial in Alzheimer's disease. Submitted.

Chapter 6

Dankwoord

Dankwoord

Een proefschrift komt niet tot stand zonder de medewerking, steun, inspiratie en aanmoediging van vele anderen. Deze personen zou ik dan ook allen willen bedanken en een aantal daarvan in het bijzonder.

Allereerst wil ik de patiënten met de ziekte van Alzheimer en hun naasten bedanken voor de deelname aan mijn onderzoek. Zonder u was dit boekje er niet geweest. Door de vaak openhartige gesprekken die we gevoerd hebben, heb ik veel geleerd over de impact van deze ziekte op het dagelijks leven. Dit heeft een diepe indruk op mij gemaakt. Hoewel mijn aandacht nu verschoven is in de richting de epilepsie, zal ik de zoektocht naar een medicijn dat deze ziekte kan genezen altijd op de voet blijven volgen. Ik hoop dat dit niet lang meer zal duren.

Vervolgens mijn promotor, prof. dr. H.P.H. Kremer. Beste Berry, bedankt voor het onuitputtelijke enthousiasme voor mijn onderzoek en het vertrouwen dat het allemaal wel goed zou komen, ook op de momenten dat het onderzoek en/of mijn motivatie in een diep dal verkeerde. De snelheid waarin jij de eerste versies van mijn artikelen rood deed kleuren was indrukwekkend. Ik heb veel van je geleerd, niet alleen op het gebied van wetenschappelijk onderzoek, maar ook tijdens mijn opleiding.

Verder gaat mijn dank uit naar dr. R.W.M.M. Jansen, een van mijn twee copromotores. Beste Rene, je nuchtere inbreng tijdens de besprekingen en je invalshoek vanuit de klinische geriatrie zijn van essentieel belang geweest voor mijn onderzoek. Het multidisciplinaire werken op de geheugenpolikliniek, waarbij de patiënt centraal staat, is de inspiratie geweest voor mijn huidige functie.

En dan mijn tweede copromotor, dr. ir. M.M. Verbeek. Beste Marcel, op het moment dat duidelijk werd dat het afronden van het klinische deel van mijn onderzoek nog lang zou gaan duren, heb je mij de kans gegeven mij te verdiepen in de liquordiagnostiek bij dementie. Je indrukwekkende kennis op dit gebied en je geweldige humor hebben er voor gezorgd dat ook dit deel van mijn onderzoek tot een mooi einde is gekomen.

Alle medeauteurs van de artikelen en personen, die op een of andere manier een bijdrage aan de artikelen hebben geleverd, wil ik graag bedanken. In het bijzonder wil ik bedanken George Borm, voor de statistische ondersteuning, Arenda van Beek, voor het opzetten van de database van geheugenpolikliniek patiënten, David Burger, voor de farmaceutische ondersteuning en de analisten van het neurochemisch laboratorium voor het analyseren van de liquormonsters.

Tevens wil in de afdeling Neurologie en met name mijn opleider prof. dr. G.W.A.M. Padberg bedanken voor de gelegenheid die ik gekregen heb om dit onderzoek te doen. De afdeling Geriatrie ben ik zeer erkentelijk voor de gastvrijheid en behulpzaamheid.

De (oud-) assistenten neurologie wil ik bedanken voor de gezelligheid en de saamhorigheid. Ik heb veel goede herinneringen aan mijn tijd op de vijfde verdieping.

Verder ben ik heel erg blij dat mijn oud-carpool genootjes mijn paranimfen willen zijn. Lieve Mariëlle, we hebben samen ongeveer 60.000 km afgelegd en tijdens de reistijd (die steeds maar langer werd) lief en leed gedeeld. Lieve Anouk (of Noekie, zoals mijn oudste dochter je noemde), ook met jou heb ik vele gezellige kilometers afgelegd, wat het reizen een stuk aangenamer maakte. Heel erg bedankt dat jullie mij op deze belangrijke dag willen bijstaan.

Dan zijn er nog vele vrienden, die al die jaren belangstellend de vorderingen van mijn promotieonderzoek hebben gevolgd. In het bijzonder wil ik Leontien bedanken voor haar nimmer aflatende interesse gedurende dit lange traject.

Mijn huidige collega's van de Hans Berger Kliniek wil ik bedanken voor de aanmoediging en de gelegenheid die ik heb gekregen om mijn boekje af te ronden.

Mijn schoonouders, Diny en Fedde, wil ik bedanken voor het oppassen, zodat ik tijd had om aan mijn onderzoek te werken.

Uiteindelijk was ik nooit zo ver gekomen zonder de onvoorwaardelijke steun en liefde van mijn ouders. Lieve mama, heel erg bedankt hiervoor. Wat is het jammer dat papa dit niet meer mee kan maken; hij zou ongetwijfeld vreselijk trots zijn geweest.

Tenslotte wil ik het thuisfront bedanken. Lieve Niels, heel erg bedankt voor je steun en "sponsoring" in de afgelopen jaren. Wat ben ik blij met jou en onze meisjes! Lieve Josefien en Feline, bedankt voor alle heerlijke momenten naast het werk, die het moeizame traject van een promotieonderzoek relativeren. Uiteindelijk is "mama" een nog mooiere titel dan doctor.